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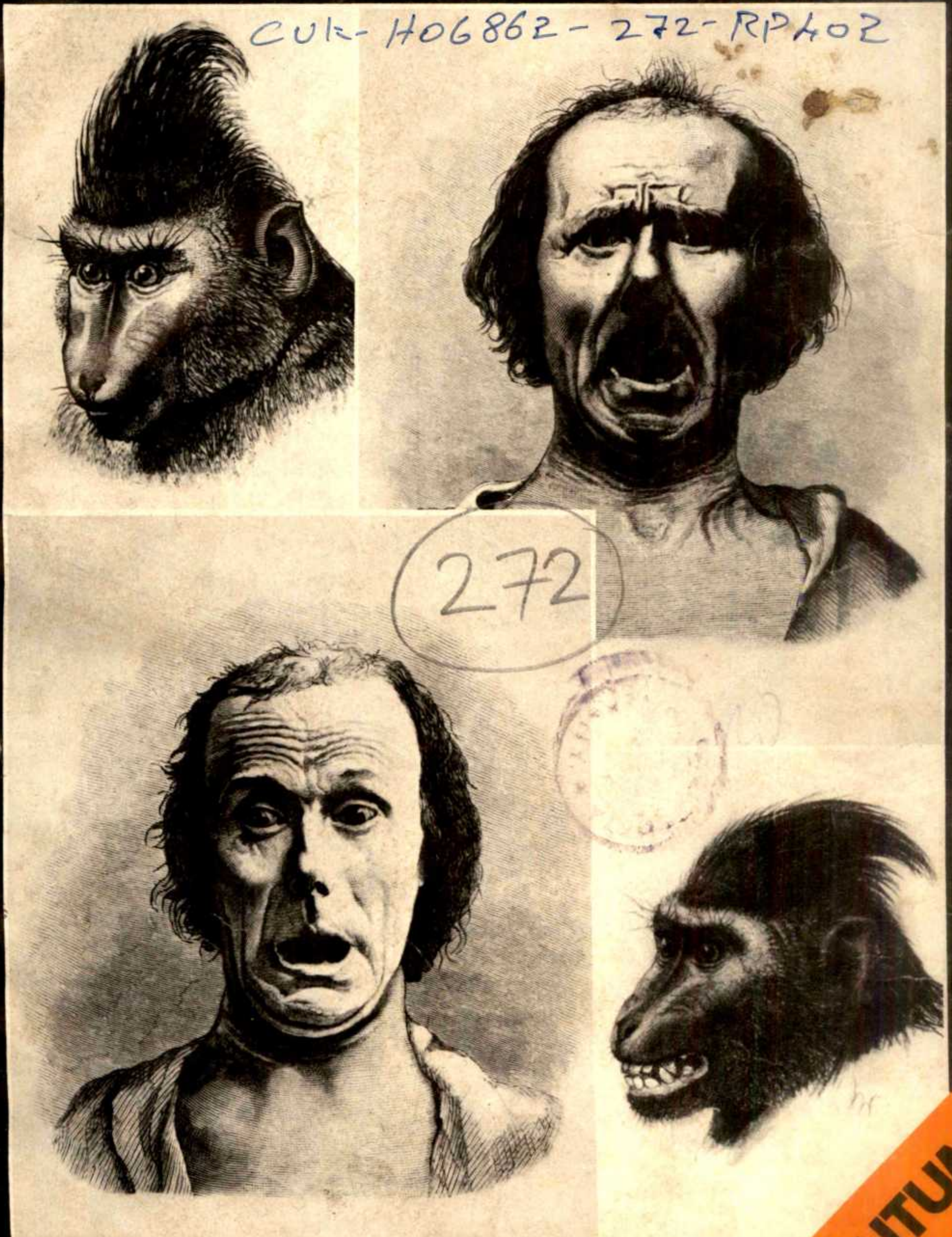

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Now reason can prevail

Sir Gordon Wolstenholme, Chairman of Britain's Genetic Manipulation Advisory Group, said on London Weekend Television last Sunday that GMAG's new system of guidelines for assessing the risks of genetic manipulation experiments "will be so full of sense in the end that it will be the one which will predominate." GMAG has not yet formally adopted the system (which is based on a version of numerical risk analysis) and has opened the subject for debate with the article below (p 104).

The revised guidelines proposed by the US National Institutes of Health are in many ways arbitrary. They are thus open to bitter public attack. The new GMAG system, on the other hand, is rational and therefore more defensible; and it is flexible in that experiments on risk are encouraged and new data can easily be incorporated. GMAG should be encouraged to adopt the new system. But GMAG's path is not strewn with roses.

First, there are internal problems. While the procedure outlined below is clearly rational, it still leaves many of the cards in GMAG's hands. 'Qualitative' biological judgements have to be made alongside the calculations, and it would help if GMAG were to be more explicit about these judgements. It would help also if GMAG's existing case law—which will still be applied where data is not available to support the more scientific system—were to be published.

It would also help if the history of the more numerical parts of the procedure were known. Sydney Brenner had the original idea of calculating a number of separable 'risk factors' for each experiment (last week he said: "I don't believe anything until I see numbers"). His idea was developed in the Safe Vectors Sub-Committee of GMAG, where a trial was run of numerical assessment. Individuals were given a list of experiments whose risks were to be calculated; but they came up with very different figures. However the *ranking* of the experiments with respect to risk was highly consistent, and hence the stress on the use of the figures for rank rather than as absolute numbers in the GMAG document. Also, there is no mention of a 'scale-up' factor in the report. The system is flexible enough that one could be included, but it is not the easy matter it might at first seem. As one GMAG member said "which would you be more afraid of: an experimenter dropping a one-litre glass flask, or an escape from a sealed stainless steel industrial plant containing 10,000 litres?" These and other matters should be aired in the debate to follow, and the new procedure should become better understood and refined in the process.

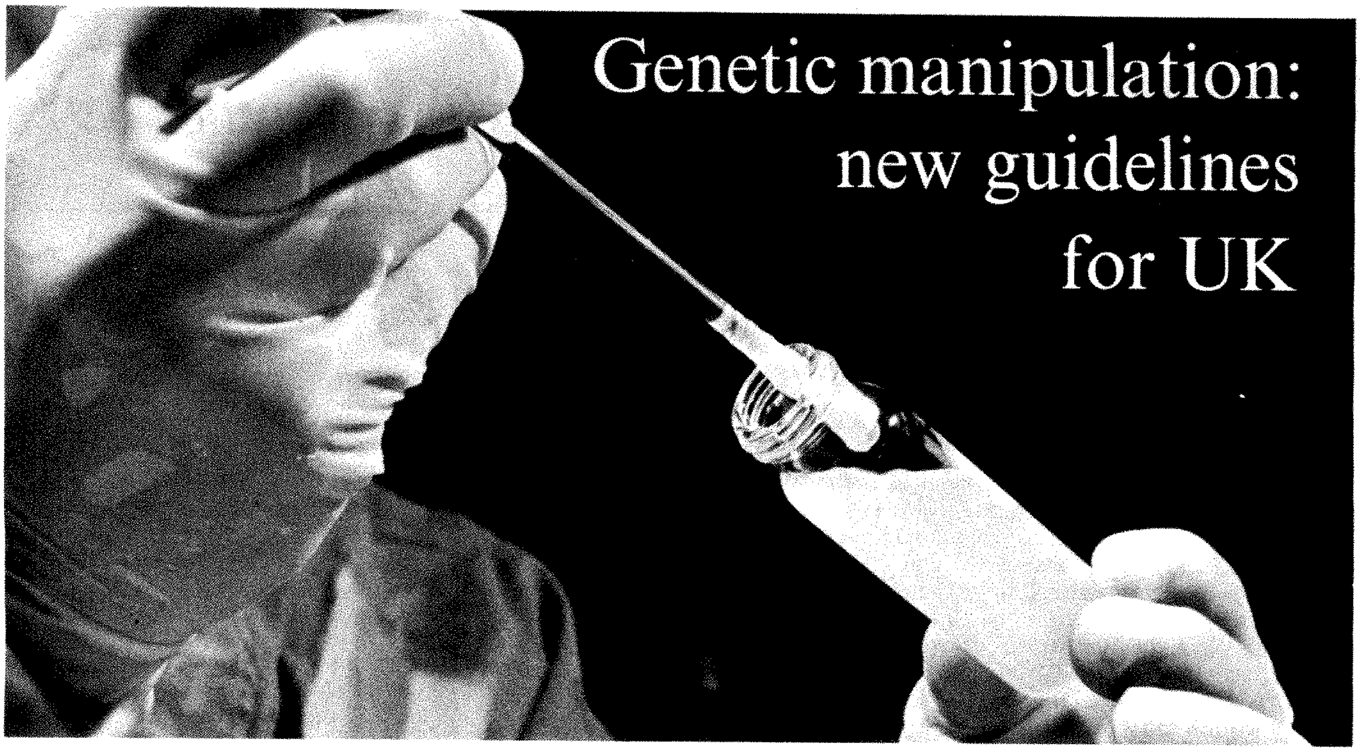
The second problem GMAG faces is international. GMAG—unlike the National Institutes of Health in the US—controls industrial research as well as academic work. So—certain industrialists argue—British firms will be penalised in the early stages of the potentially enormous business of biotechnology if GMAG's controls are any stiffer than those adopted voluntarily or by fiat elsewhere. It is important for industry that the controls on this research are internationally uniform. But the NIH guidelines, for example, take no care of what GMAG calls the 'expression factor'—whether the foreign gene is translated into protein or not. As industry clearly wants expression (that gives it its product) it is to industry's advantage that that factor is ignored. And this is just one example. To make such things uniform internationally is no easy matter, and it is important that it should be pursued at the highest government level.

International uniformity becomes a further cause why GMAG must make its procedures crystal clear. The European Science Foundation's Liaison Committee on genetic engineering adopted the earlier British (Williams) guidelines used by GMAG; but its members were lost in applying them to their own countries. What exactly were GMAG's internal procedures? And how could the smaller countries apply them, where there were too few researchers to set up a GMAG equivalent? The NIH system has won in many countries for its transparency—its lock-up table of containment—despite its evident logical shortcomings. Europe needs a European GMAG. Perhaps the ESF Liaison Committee itself could take on the role?

The third problem facing GMAG is the research on risks it so welcomely recommends. How is it to be done? Who is to do it? And who pays? Surprisingly, perhaps, the last question is the easiest to answer. Britain's Minister of State for Education and Science, Mrs Shirley Williams, interviewed last week on London Weekend television by MP Brian Walden, promised "tens of millions of pounds" for risk research in Britain. With that much, risk research might become like cancer research in the US: everybody will be doing it because the money is there. £10 million would certainly answer the previous question—who is to do it?

There remains the problem of careers for 'riskers'; as Brian Walden put it "there are no Nobel prizes for risk research". (Though who knows? One of the risks is of the escape of a bacterium producing large amounts of insulin, and no-one knows what such a bug would do. Another risk is of a bacterium expressing, say, cell surface proteins upsetting the immune system and inducing an autoimmune disease. Again, too little is known about the immune system to predict the consequences. Experiments on such matters might throw up new knowledge.) But if university researchers cannot be drawn away from their present interests what of the civil service scientists? What about those ex-Ministry of Defence people at Porton Down, now under Shirley Williams' control? Could they begin work on risk research?

Then there is also the problem of how risk research is to be commissioned. Surely GMAG should do that. GMAG has already expressed a willingness to do so, through the 'technical panel' GMAG recommends in its new proposals. And one final point. That panel should not consist entirely of academics, as its name appears to suggest. One of GMAG's strengths has been the breadth of interest represented by its members. It should not abandon that strength in what will become its inner sanctum. ☐



Genetic manipulation: new guidelines for UK

● Britain's Genetic Manipulation Advisory Group (GMAG) is preparing to adopt a new approach to assessing the risks of 'genetic engineering' experiments. GMAG's statement —approved in principle at a meeting on 2 November—appears below. The scheme is based on an attempt to assign numerical risks to individual experiments as a product of three factors: the 'access factor', the 'expression factor', and the 'damage factor'. But the numbers are used only to rank the risks so that the experiments can be assigned consistently to the containment categories I to IV. Qualitative matters are also taken into account, for biology has not yet been reduced entirely to numbers.

The scheme will be used when there are enough data to employ it; otherwise assessment will revert to the old Williams 'phylogenetic' guidelines. Its great virtue appears to lie in its objective scientific basis and its consequent encouragement to research on risks.

GMAG's Sub-Committee on the Validation of Safe Vectors* and, more recently, an *ad hoc* Working Party on Guideline Criteria†, have been considering on GMAG's behalf the merits of an alternative approach to the categorisation of genetic manipulation experiments first outlined by Dr Sydney Brenner (MRC Laboratory of Molecular Biology, Cambridge). GMAG, having considered the enthusiastic advice it has received, is persuaded of the general soundness of the new system, and subject to the consideration of comment on this discussion document and consultation with the Department of Education and Science and the Health and Safety Executive, hopes to introduce the new principles—to be used for the time being in parallel with the existing Williams Guidelines[1]—early in 1979. Before refining the system, GMAG wishes to take into account the opinions of the scientific community, and it is therefore hoped that publication of the following will allow all concerned to make their views known to the Group‡. In the very near future, GMAG will be arranging a meeting with users and with members of local Biological Safety Committees.

While seeing justification and opportunities for change, GMAG considers that there is no case for changing the general arrangements governing this area of research in the UK: the definition of genetic manipulation remains unchanged; the regulations[2] to notify work being done must apply; the roles of GMAG and HSE have been established; facilities for physical containment (categories I-IV) have been defined and substantial investment has gone into their construction.[3] Proposals are put forward in this paper, however, which if implemented would lead to change in the scientific considerations upon which the categorisations of individual experiments are decided by GMAG.

At present, GMAG broadly follows the Report of the Williams Working Party in allotting experimental protocols to the four categories of physical containment. The chief conclusion of the arguments which follow is that those experiments for which it is possible to carry out an approximate risk assessment should where appropriate be given categorisations distinguished by the labels I*, II*, III* and IV*. (It must be emphasised that no change is implied in the specification of physical containment facilities, but only in the *categorisation* of certain experiments.) It is suggested that the two systems of categorisation should persist in parallel for as long as may be necessary. At this stage relatively few experiments can be given the new starred categorisations, but a steady transfer of experiments and classes of experiments from one system to the other should be possible.

The Williams guidelines

Although in the past two years GMAG has based its advice on the "suggested categorisations" of the Williams Report, there have been several occasions when it has been necessary to modify or to extend that scheme. That this should have been so is not surprising, for the Williams Working Party reported when there had been very little practical experience of genetic manipulation. Moreover, the report explicitly acknowledged that its categorisations were likely to be found insufficient in some respects, and it

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**Professor J. H. Subak-Sharpe (Chairman), Dr S. Brenner, Professor D. C. Ellwood, Dr M. Fried, Dr M. Moss (Secretary), Professor K. Murray, Dr N. Murray, Professor M. H. Richmond, Dr P. W. J. Rigby, Dr R. Weiss, Dr N. Wilkie, Professor R. Williamson.*

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foresaw the need for refinement and substantial modification.

First, doubts have accumulated about the value of the criterion of phylogenetic relatedness as a measure of the possibility that genetic manipulations may be hazardous. A simple illustration will show the difficulty. According to the suggested categorisations, genetic elements from plants entail less relative hazard (to human beings) than genetic elements from higher organisms, yet it is also clear that the incorporation into the genome of a strain of *E. coli* viable in the human gut of a gene responsible for a plant immunogen (say the glycoprotein gluten from wheat germ) could be relatively more hazardous than a gene responsible for the production of, say, human globin. More generally, the bearing of phylogenetic relatedness on the potential dangers of experiments in genetic manipulation must be modified by the nature of the host and the vector; animal genes are, for example, less likely to retain their original functions when incorporated in the genomes of bacteria than in those of animal cells or viruses.

Second, the experience of the past two years has also shown that there is a need for a modification of the Williams prescription about the identity and the purity of transplanted genetic elements. Often the more relevant characteristic is whether the foreign gene is capable of being expressed (or otherwise retaining its original function). Moreover, evidence has accumulated in the past two years to suggest that many of the genes of higher organisms are distributed as separate pieces among the genetic material of the donor organism, which decreases the likelihood that shotgun experiments in which pieces of random DNA are transferred to other (and especially bacterial) hosts will yield functional genes in the new genetic environment. On the other hand, experiments in which genetic elements are obtained in potentially functioning form from functional molecules of messenger RNA, often regarded in the past two years as an assurance of their purity, may be in principle *more* potentially hazardous than the same genetic elements obtained directly from the genomic donor DNA. These statements are not, of course, of general application, but they are another reason to believe that individual cases should be dealt with separately.

Considerations of the host-vector system remain generally applicable. If there are hazards in any experiment in genetic manipulation, it will plainly be beneficial that a host organism carrying foreign genetic elements should be incapable of infecting human beings, and that the vectors used in the experiments should be specific to these host organisms. Appropriate use of "disabled" host-vector systems, offering "biological containment", will continue to be one of the essential features of certain types of recombinant DNA research.

More objective methods

In the circumstances, it is likely that all interested parties, scientists and the general public alike, would benefit if there were a more objective and even quantitative basis for assessing the risks of genetic manipulation. But it will be some time, perhaps several years, before biological understanding and quantitative data have accumulated sufficiently for such techniques to be applied to all proposals for experiments in genetic manipulation.

Elements of risk assessment

Assessment of the risk of experiments in genetic manipulation must follow a formal and comprehensive risk analysis, which is possible only when the biology of the host-vector system and of its interaction with the cells of organisms at risk is reasonably well known. At present the relevant biological data are not generally available, and many systems of potential value in genetic manipulation will therefore be excluded from the categorisation procedure now proposed. With the passage of time, however, it will be possible to include within the new basis for categorisation an increasing proportion of experimental proposals.

In general, risk analysis entails a consideration of the following steps.

(a) *Escape from containment*

The escape of organisms carrying foreign genetic elements from the containment specified for them is always a possibility, either because the facilities are not designed in such a way as to provide absolute assurance that organisms will not escape or because of some malfunction or accident. Thus it is proper to associate with each category of containment a specific probability (C_i, where i runs from I to IV) that organisms will escape. The quantities C_i have the units "probability per unit time per organism contained".

The ratios of these probabilities will be measures of the relative efficacy of the four different levels of containment, and there are at present only rough estimates of the ratio C_{IV}/C_I, the relative safety of the most stringent and the least stringent of the containment categories now in use. The ratio is likely to be less than 10^{-6} (corresponding to a factor of 100 for the interval between each category of containment) and may be less than 10^{-9}. It is important that the true range of safety offered by the four categories of containment should urgently be defined more precisely. On present indications, the range of efficacy spanning the least and the most stringent categories of containment is comparable with the degree to which the viability of some host organisms is reduced relative to the wild-type strains; this points to questions for the future about the relative protection from conjectured hazard of physical and biological containment.

(b) *Persistence in the environment*

The most direct concern in the assessment of the risks of genetic manipulation is that organisms carrying foreign genetic elements will gain access to the tissues of laboratory workers, but it is important that those concerned with the safety of genetic manipulation should consider the posibility that, as an intermediate step, organisms may lodge and even replicate in the laboratory into which they have escaped (as, for example, in culture dishes). In this and other parts of the analysis, care must be taken to identify all the possible circumstances in which organisms carying particular foreign genetic elements may be relatively at an advantage, so that the genes concerned are amplified and not attenuated.

(c) *Access to organisms at risk*

Organisms which escape as aerosols may be inhaled by laboratory workers or ingested, thus gaining access directly to the nasal tracts, the lungs and the gut. It is important that unfamiliar routes of access should not be overlooked—for example, the effects on human lungs of the inhalation of *E. coli* organisms carrying genes capable of producing, say, insulin are at present unknown.

(d) *Replication*

The risk analysis of experiments in genetic manipulation differs from that of, say, the operation of nuclear power plants in that agents which may potentially cause damage may in some circumstances be capable of replication. In the risk analysis of experiments in genetic manipulation, great care should therefore be taken to identify pathways that permit the replication of organisms carrying foreign genetic elements or which permit the transfer of the foreign genetic elements to other organisms which are capable of replication. One factor in a quantitative measure of a particular hazard would no doubt be provided by the duration and the intensity of the exposure of susceptible cels to organisms carrying potentially damaging genetic elements, and it is theoretically possible to calculate quantities such as these, given assumptions about the viability of manipulated organisms in the environments in which they lodge. Such formal calculations are however unlikely to be

Examples of the new categories

- The cloning of pure mammalian globin genes in an approved disabled *E. coli* host-vector system in circumstances in which the globin genes are not expressed would be I*. For the time being, the use of non-disabled strains of *E. coli* (including K12) or the expression of the globin genes in a disabled host-vector system would place the experiment in category II*. The cloning of expressible globin genes in a non-disabled host-vector system would, for the time being, be a category III* experiment. The allocations to these higher categories are justified by the present lack of certainty that the secretion of mammalian globin in the body tracts (including lungs and the genito-urinary tract) to which *E. coli* has access would be without risk. As this uncertainty is removed, lower categorisation may be considered appropriate.

Category I* is justified for the cloning of non-expressible globin genes in a disabled host-vector system on the following grounds—all three of the factors *A*, *E* and *D* are small (notionally less than unity). If it emerges that *D* is small even when globin genes are expressed, the experiments provisionally allocated to II* and III* might be reduced in categorisation.

This experiment may therefore be considered typical of those destined for Category I*. It should be noted that two of the three factors afford a measure of biological protection, and that in spite of the apparent innocuousness of globin, GMAG would probably be unwilling to agree that an experiment of this kind should be undertaken with wild-type *E. coli.*

- The cloning of genetic elements from the genome of the mouse with the use of defective polyoma virus as a vector, and with cultured mouse cells as hosts (with appropriate helper virus to facilitate the replication of the virus and the expression of functionable genes) would also

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meaningful in present circumstances, so that more qualitative comparisons must at present suffice.

Great care must be taken to ensure that full allowance is made for the unavoidable incompleteness of present knowledge of the behaviour of foreign genetic elements in their hosts. Thus allowance must be made for the possibility that a "disabled" strain will revert (by genetic mutation) to the wild type, or that a foreign gene will be transferred (by the mobilisation of the plasmid) to wild-type bacteria. As things are, sufficient numerical data do not exist bearing on the chance that such events will occur, so that, for some time to come, researchers will have to rely on order-of-magnitude estimates of some of these quantities.

The conjectured hazards

Concern that genetic manipulation may entail hazards for living things stems from the possibility that foreign genetic elements may affect the normal functioning of particular groups of cells or the coordinated functioning of organisms at risk. Another of the impediments to formal and quantitative risk assessment that is likely to persist for some time is that of comparing the possible (but still hypothetical) biological consequences of foreign genetic elements with the damage done by naturally occurring disease. Even so, a tentative listing of some of the possible interactions between foreign genetic elements is suggestive of some of the points to which researchers should direct attention.

(i) Cell death

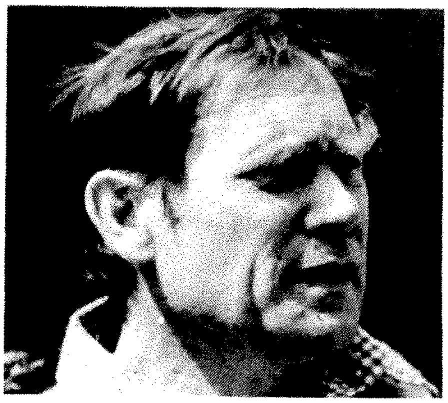
Cells may be killed when they are infected by viruses or when they are exposed to toxins produced by bacteria. Specific microorganisms and viruses often affect specific groups of cells in the body of a higher organism—poliomyelitis kills people because the poliomyelitis virus kills cells in the central nervous system when it has access to them, for example. In the analysis of the risks of genetic manipulation, the questions arise whether bacteria or viruses carrying foreign genetic elements are more virulent than the originals, whether the range of their specificity has been extended and whether bacteria carrying the intact genomes of pathogenic viruses make it possible for cells at risk to be exposed to potentially damaging viruses by unnatural routes.

(ii) Changes of genetic constitution

The introduction of foreign genetic elements into human somatic cells could have a number of harmful consequences. The state of differentiation of the cell might be affected. Cryptic viral elements already included in the DNA of the cell might be mobilised, or the foreign genetic elements themselves might be incorporated in the genome of the intact cell, increasing the range of cryptic viral function embodied in the cell. The chief causes of anxiety on such grounds have so far been in connexion with the occurrence of cancer, but understanding is far from complete. Experiments in which animal viruses multiply in human cells (in tissue cultures) without killing the cells may deserve special attention.

(iii) Immunological consequences

There are several hypothetical mechanisms whereby untoward immunological reactions might be provoked by foreign genetic elements. Thus foreign genetic elements might be incorporated in the genome of a human cell in such a way that the product of that gene (possibly a protein) was expressed on the cell surface, thus exposing the cells affected to attack by the body's immunological defences; this may correspond to the mechanism of some naturally occurring autoimmune diseases. On the other hand, the introduction of foreign genetic elements might be accomplished in such a way that their presence simulates that of the normal components of body tissues, with the result that the range of the sensitivity of the immune system would be narrowed. In principle, it is also possible that some of the cells of the immune system are especially vulnerable to organisms carrying particular genetic elements.



Sydney Brenner

(iv) Disturbances of endocrine function

Many hormones are believed to exert their physiological effects by interacting with specific biochemical elements either on cell surfaces or in the nuclear DNA. Foreign genetic elements could in principle interfere with either of these reactions, but it is also the case that infection by bacteria carrying genes which direct the synthesis of human hormones *and* which are expressed and then secreted into the body could be damaging to the individuals concerned.

(v) Disturbances of cell metabolism

Foreign genetic elements inadvertently introduced into somatic cells could in principle disturb the metabolism of these cells, changing their metabolic rate, the rate of cell division or even the process (still unknown) by which somatic cells age.

(vi) Consequences for microorganisms

Experiments in genetic manipulation in which bacteria might artificially be en-

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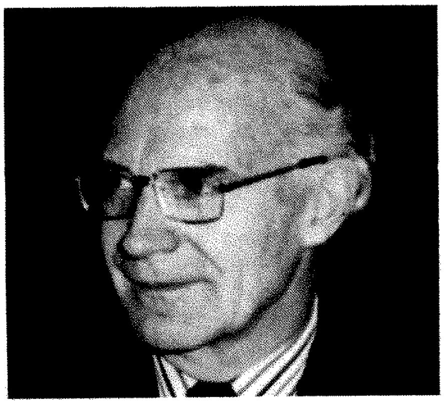
be a category I* experiment. Formally, such experiments have much in common with those in which fragments of the *E. coli* genome are redistributed within the same organism with the help of *lambda* bacteriophage. In reality, however, the possibility that polyoma virus may replicate in cells other than those of the mouse cannot be entirely discounted (replication in rat cells has been demonstrated, while the possibility that polyoma might replicate in human cells has not been vigorously explored). Accordingly, the cloning of *pure* mouse genes in polyoma and cultured mouse cells is suggested as a category I* experiment, with mouse-genome shotgun experiments in category II* for the time being.

- Category I* will also include those experiments for which at present there is no substantial evidence of risk. The chief candidates of this kind are the experiments in which *E. coli* genes are cloned in *E. coli* organisms.
- If these illustrations are taken as a paradigm of experiments in category I*, then it would follow that disabled *E. coli* carrying but capable of expressing the gene for, say, wheat germ gluten would be categorised II* to allow for the possibility that at least some members of the population might react immunologically against the production of gluten in such a way. Other experiments that might be given the same categorisation on further consideration by the technical panel might include the incorporation of non-expressed interferon genes in *E. coli* K12, or expressed interferon genes in a more disabled strain of *E. coli* K12 (eg χ1776). Shotgun experiments with mouse DNA cloned in polyoma virus would also be included.
- Similarly, and again on further examination by the technical panel, category III* might include disabled *E. coli* strains carrying expressed human growth hormone genes; the expression of cholera toxin genes in disabled strains of *E. coli* would remain in category IV*.



Sir Gordon Wolstenholme

dowed with resistance to antibiotics of medical or veterinary importance are potentially hazardous. Another early suggestion of a damage mechanism is the possibility that apparently innocuous organisms (such as the laboratory strains of *E. coli* K12) could be converted by genetic manipulation into infectious pathogens; relevant in this regard is the conclusion of a conference held at Falmouth (New Hampshire, USA) in 1977 that *E. coli* K12 at least cannot be converted to an epidemic pathogen. There are three kinds of reasons for this conclusion: the gentic basis of pathogenicity is complex and does not reside in a single gene, the wild-type strains of *E. coli* which have evolved are probably in some sense optimal for the ecological niches they occupy and the viability of laboratory strains of *E. coli* is so much less than that of wild-type bacteria that such organisms carrying pathogenic elements would probably be eliminated rapidly. This conclusion does not imply that individuals could not be damaged by *E. coli* carrying pathogenic elements such as the gene for cholera toxin, but merely that the production of an *infectious* pathogenic agent is improbable. There is a need for a consideration along the same lines of other organisms capable of infecting human beings.

(*vii*) *Ecological consequences*
Similar considerations apply to the interaction of foreign genetic elements and species other than human beings. To the extent that species were differently affected and that foreign genetic elements were widespread, ecological changes are in principle possible. Changes in the ecological balance of microorganisms are perhaps especially important.

For those assessing recombinant DNA experiments, this list should make it possible to single out those kinds of interactions between foreign genetic elements and cells which are likely to be widely regarded as potentially serious and damaging. Possible immunological and oncogenic consequences point to the need for a relatively high category of physical containment.

Application in the immediate future

In an ideal world, it would be possible to make numerical estimates of the risk of experiments in genetic manipulation by multiplying together the probabilities representing the various events (*a*) to (*d*) (see page 105), summing over all possible pathways leading from the escape of a genetically altered organism from its containment to the causation of a particular consequence for cells exposed and then multiplying by factors which take account of the costs of the damage that results. This is not now possible, however. Some may even argue that it will never be possible or necessary.

Nevertheless it is possible to use the framework of this formal risk assessment to rank the relative risks of some experiments in genetic manipulation, albeit in a somewhat qualitative manner. To that extent, the arguments first put forward by Dr Sydney Brenner in the GMAG Safe Vectors Subcommittee are a valuable means of moving towards a more objective system of categorisation.

What is now proposed is that experiments with which researchers are familiar should be allocated to the four containment categories on the basis of a qualitative and relative assessment of their risks; and that the potential risks of novel experiments should then be assessed in relation to these familiar paradigm experiments.

For this purpose, it is convenient (as Brenner has argued) to define a group of three factors which may be taken to represent the chance that events following the escape of genetically altered organisms into the environment may lead to particular conjectured hazards. If data were available, it would then be possible to think of calculating the risk of an experiment by multiplying the numerical values of the three factors together. For the time being, however, such a procedure would provide a spurious sense of precision and it is proposed that there should be a comparison (which necessarily entails the exercise of people's judgment) of the values of the three factors relative to those which obtain for the paradigm experiments.

Analysis might therefore proceed as follows. First, it is supposed that there is an escape of organisms carrying foreign genetic elements. The overriding element in the assessment of the risk of a particular kind of disturbance is the likelihood that the organisms or the genetic elements they carry will have access to susceptible cells of the target organisms. Thus there is defined an

- **Access factor** (*A*). This is plainly a composite of factors representing the chance that escaped organisms will actually enter the human body, that they will survive there and that they will penetrate whatever membranes must be penetrated so as to reach the tissues containing susceptible cells. For many experiments, biological containment is dominant—for example the disabled strain of *E. coli* K12 known as χ1776 may be less viable than wild-type strains of *E. coli* by a factor of 10^8.

In some experiments in genetic manipulation, the potential risk depends crucially on whether the foreign genetic element is able to express its normal function. This would, for example, be the case in an experiment in which the gene specifying the cholera toxin was incorporated in a plasmid of *E. coli*. Thus it is convenient to define an

- **Expression factor** (E), which would be large (or notionally have the numerical value 1) if the foreign gene were efficiently translated into the protein product of the gene and if the products were then secreted from the altered organism, which would have a similar value in those circumstances (as for example in the carriage of a genetic element which can be integrated into the genome of target cells without being translated) but which would be small in those experiments in which steps had been taken deliberately to ensure that the gene would not be expressed.

Shotgun experiments require further consideration. The fear that "unknown" genetic elements might cause unknown and damaging changes in organisms at risk has been widespread for the past four years. The Williams Report suggested that experiments involving random lengths of DNA should be categorised somewhere in between the categorisation appropriate for genetic elements known to be damaging (eg cholera toxin) and those thought to be without risk. This conclusion remains valid, but knowledge of the genetic constitution of organisms is growing quickly, and for some it is now reasonably certain that a genetic element drawn at random is likely to be less of a relative hazard than one deliberately selected. This, for example, may be the case with many animal viruses, whose genetic structures appear to be so economical of DNA that it is hard to believe that a piece drawn at random would have physiological significance. Thus it is convenient to define a

- **Damage factor** (D), which expresses the chance that a genetic element will cause damage. D will be large (notionally, unity) if, for example, the host organism were known to contain a gene specifying a bacterial toxin; will be less if it is a genetic element drawn at random from the same bacterial genome; and will be very small if the product of the gene is known to be without physiological effect on the cells of the target organism (as may be the case where the gene product is, for example, human globin).

It is proposed that, in the months ahead, GMAG should take steps to allow researchers (if they wish) to submit their proposals to GMAG with evidence which will allow GMAG to make an assessment of the three factors and advise on the new basis. Given the present incompleteness of biological understanding and the lack of data bearing on the numerical determination of these factors, it is clear that, for the time being, decisions will continue to depend on judgment rather than calculation. It is also clear that experiments using familiar systems will be the most susceptible to this alternative treatment. One advantage of following such a course of action will nevertheless be to encourage the accumulation of data bearing on the ultimately more objective assessment of risks.

Proposals

The particular recommendations that follow are designed so as to achieve the following objectives. First, the intellectual framework of risk analysis should be used as a means of further refining the self-consistency of GMAG's present procedures. Second, GMAG's procedures should remain flexible, and be capable quickly of being adjusted in the light of new knowledge. Third, new criteria for deciding on categorisations should be applied at the outset only to the systems with which researchers are most familiar, with the expectation that, with the passage of time, increasing numbers of experiments will be included.

It is therefore proposed that GMAG should take the following steps as soon as this may be convenient:

1. The present Williams categorisations will be retained, and GMAG's present methods of assessment will be retained for experiments whose relative hazards cannot be assessed in the manner outlined above. But researchers will also be invited to ask that experiments should be given a different categorisation than suggested by Williams if they have reason to believe that in their case the factors A, E and D would justify such a course. Similarly, GMAG should be able to require more stringent categorisation in appropriate circumstances.
2. GMAG will set up a technical panel to scrutinise such applications on the alternative basis and to report to GMAG, to collect data bearing on a more thorough understanding and evaluation of the factors A, E and D, and to publish (through GMAG) to the scientific community advice on the continuing refinement of the procedure. Researchers might have to accept that, initially at least, these complementary procedures might entail some delay.

   In the first instance, it would be for applicants to provide such empirical data as they can on which the technical panel's consideration of the three factors would be based. It is hoped, however, that the technical panel would be a powerful stimulus of the research needed to make risk assessment more objective and widely applicable. Refinement of present knowledge of the relative efficacy of the different categories of containment may be thought an urgent need.
3. The proposed system must be grounded by the allocation of some paradigm experiments to the four categories of containment.
4. It is proposed that for experiments of the kind referred to in the box (item (*c*)), and for others in which the genes of *Bacillus subtilis* and *Pseudomonas aeruginosa* are rearranged, it should be open to laboratories to provide a block notification of a programme of experiments to be carried out over a substantial period of time, say a year. A condition of the acceptance of such a notification will be the inspection of the laboratory by GMAG, an undertaking to work in category I* conditions and to submit a report at some required interval (not less than a year) providing a detailed log of all experiments carried out. Laboratories working to such a block notification would be required to inform GMAG of all new workers in the laboratory, and of the transfer of organisms to other laboratories; but the use of such organisms in recipient laboratories could be carried out under conditions of good microbiological practice (PHLS Handbook No. 4). In due course, this category of experiments without discernible risk will no doubt be extended.

Acknowledgments

The credit for urging on GMAG the risk assessment approach to genetic manipulation and for drawing it first to the attention of GMAG's Safe Vectors Sub-Committee goes to Dr Sydney Brenner, whose cooperation in developing the system into its present form is also warmly acknowledged. GMAG also wishes to thank the Guideline Criteria Working Party for their work in drafting the present paper, and innumerable other experts who have generously given their advice. ☐

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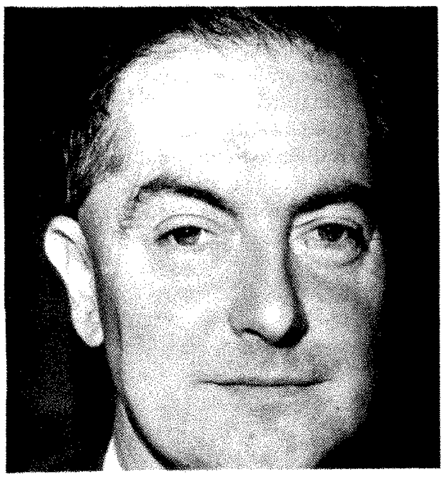

Top Scottish astronomer resigns after staffing row

Professor Vincent Reddish (above), director of the Royal Observatory, Edinburgh (ROE), handed in his resignation last week to the UK Science Research Council (SRC) which runs the observatory. When he relinquishes the directorship next September, he will also leave two associated posts, Astronomer Royal for Scotland and Regius Professor of Astronomy at Edinburgh University.

Professor Reddish's decision to resign has come after a prolonged dispute with the SRC over filling some senior posts, in particular that of head of technology. Administrators within the SRC would like to see the post go to an engineer. Reddish would like it to be given to an astronomer with some technological experience, who could interchange with astronomers at the observatory's other telescopes in Hawaii and Australia. The resulting deadlock has led to some posts remaining unfilled for years, according to Professor Reddish, and the lack of some senior staff is now beginning to impair the work of the observatory.

The SRC is trying to persuade Reddish to postpone or reconsider his resignation. It would like to readvertise the head of technology post inviting both engineers and astronomers to apply. The ideal candidate, according to an SRC spokesman, would be someone who is primarily an astronomer with a great interest in or some experience of associated engineering. Reddish feels that the reforms needed to make him change his mind are primarily too great for the SRC to implement. "In principle," he says, "the problem is essentially who decides how to do the work here and the kinds of skill needed to do it." In the event of no successful settlement, he plans to retire from astronomy altogether and move into business. ☐

Appleton and Rutherford to merge

At a meeting on 18 October, the Council of the UK Science Research Council (SRC) took the decision to close down its Appleton Laboratory at Slough and move it to the Rutherford Laboratory, also run by the SRC, at Chilton in Oxfordshire. The reasons for the merger are scientific, says the SRC. "Bringing these teams together would be the best way of doing their scientific work", according to Dr Brian Oakley, SRC Secretary.

The Appleton Laboratory is responsible for managing most of the UK's scientific space programme, which includes satellites, balloons and rockets, for scientists working in universities. The Rutherford Laboratory has always been the home-based focus of the UK effort in high energy physics at CERN. With the closure of its particle accelerator, Nimrod, earlier this year, however, it has had to diversify. It now houses a central laser facility, computing facilities and is building a central spallation neutron source.

The SRC hopes that two activities in particular, space work and radiocommunications, will benefit from the amalgamation of skills. At present work on both of these is done mainly at the Appleton Laboratory, although Rutherford has provided some engineering support for space projects in the past. Most recently, it helped design the stratospheric and mesospheric sounder for NASA's Nimbus-G satellite, launched towards the end of October. And it is currently working on the design of a millimetre wave radiotelescope with Appleton.

However some of the scientific staff at Appleton are far from content with the move. "The SRC came to a hasty decision without considering in sufficient detail all the points we would have liked them to" said Dr Jo King, Chairman of the staff side at Appleton. He feels there is little advantage to be gained from the move. Usually, he says, satellites are built in several different places and put together in one place. This would still have to happen even when the two laboratories merge. In the case of radio propagation work, Dr King claims that there will be too much radiointerference at Rutherford to make such work effective. "We are making very sensitive radio receivers" he says "and being asked to put them in one of the noisiest places in the UK".

The move is expected to take five years to complete. Appleton will be receiving data from the UK Ariel 6 satellite, to be launched next year, and the UK/NASA/Dutch satellite, IRAS, which will be launched in 1981. Final transfer to Rutherford therefore cannot be completed until those satellites cease to be operational. The bulk of the move will take place between 1980-83.

The engineering support at Appleton is weaker than that at Rutherford, according to Dr Geoffrey Manning, Rutherford's deputy-director, because it is the smaller of the two. When they are amalgamated, he says, it should be possible to provide laboratory support for all of the UK's space programme at one site. According to another SRC official, this should maximise the chances of the UK participating with the United States in the multi-mission refurbishable satellite (mrs)—a satellite proposal which has gained much support from the astrophysicists but little enthusiasm from geophysicists.

Judy Redfearn

Greater powers for Dutch science minister

The Dutch minister for science policy, Dr Marius Peijnenburg, has recently extended his role beyond that of coordinator. Together with one or two other ministers he now steers scientific research in government institutes and industrial laboratories. So far this has been the government's task.

With the minister for education and science, Peijnenburg will now be involved in decision-making at an early stage in university research as well as that carried out in the institutes of the Royal Netherlands Academy of Sciences. He will also be involved in formulating policy for the energy research centre, the aviation and space laboratory and the institute for aircraft development, which until now came solely under other ministers. Peijnenburg will also control TNO, the national organisation for applied research in the natural sciences. It has a staff of about 5,000 and an annual budget of £55m.

The 1979 research budget totals around £1.5 billion, 2.05% of the gross national product. More than £0.7 billion comes from government and £0.8 billion comes from industry (mainly from Philips, Shell, Unilever, Dutch state mines and AKZO). In 1978 and 1977 the research budget was £1.4 billion and £1.2 billion respectively. For the first time, the minister for science policy is to have his own small budget for stimulating new projects in areas such as systems analysis and technology assessment. It should increase to £3m a year by 1981.

In 1979 the time spent on non-nuclear research as a percentage of

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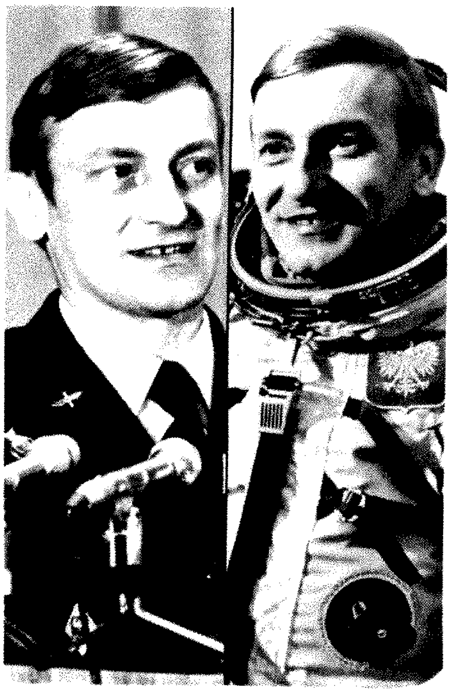
that spent on all energy research will increase from 36% in 1977 to 45% in 1981. Of the £57m spent on energy R&D in 1978, £30m is being spent on nuclear energy. Holland contributes £14m to the EEC energy research programme of which about £10m is spent on nuclear research.

Before the budget was published at the start of the new parliamentary year, the ministry for science policy announced that the Science Information Bureau will become permanent and be expanded slightly. It has been operating on a trial period for two years at the Academy of Sciences. And at a meeting of the special parliamentary committee for science policy at the end of October, the minister announced the setting up of a group of experts to study the effect of microelectronics, especially microprocessors, on society. The group should report within three months. **Casper Schuuring**

Poland tries the taste of space

During 1978, three cosmonauts from Eastern Europe visited the Soviet Salyut-6 orbiting space station. **Vera Rich** talks to the Polish cosmonaut, Miroslaw Hermaszewski in Warsaw



Two faces of Cosmonaut Hermaszewski; left—meeting the press; right, set for space.

MIROSLAW Hermaszewski, like his two brothers, is a pilot by profession, not a scientist. However as a cosmonaut he was obliged to fulfil a dual role—crew member and scientific worker. In the latter capacity Hermaszewski was responsible especially for space medicine, which he describes as "a Polish speciality".

The medical programme concentrated on two experiments: "kardiolider" and "smak". The first monitored the cardiovascular system "to determine the potentialities of the heart in space and also during pre-flight training". This, Hermaszewski told me, meant that "from the psychological point of view we felt very safe, knowing our hearts were being constantly monitored, especially when we were not in contact with Earth". (Constant contact, he explained, is not maintained as a rule, although Soviet ships around the world are on constant emergency watch.)

The second medical experiment "smak" (Polish for "taste") was designed by the Academy of Military Aviation Medicine to monitor sensory changes under conditions of weightlessness. A few days before take-off, the cosmonauts sampled various menus and chose those they wished to take into orbit. "We chose the meals we preferred" said Hermaszewski "but after a few days in space we didn't like them. This means that our sense of taste had changed."

Challenged that the falling off of appreciation could be due to deterioration of the rations and/or boredom, Hermaszewski explained that the meals were specially prepared to preserve quality and taste, and also that there was "a menu-cycle of six days". "And even then, it wasn't exactly the same meal", he added. "I mean, if on one day we had, say, *barszcz* and turkey at one meal, then six days later we'd have *barszcz* and turkey again, but not at the same meal." The scientific team who worked out the experiments had, he said, developed a numerical method of estimating taste, which allowed the activity of the taste-buds to be monitored quantitatively.

Both "smak" and "kardiolider", added Hermaszewski, were important for medicine in general. Versions of kardiolider were now being produced for use in post-coronary rehabilitation programmes, while "smak" could provide valuable data about sensory processes.

With the third main Polish experiment, 'Syrena', Hermaszewski clearly felt his responsibilities considerably. Although he worked in the experiment with the Soviet cosmonaut, Ivanchenkov—one of the long-stay Soviet crew—he was aware that the experiment had been developed by Polish scientists. "It was absolutely Polish from the beginning, and was called 'Syrena' after the symbol of Warsaw". He admitted that during the running of the experiment which took 5 to 6 hours each time, he kept getting up at night to check the equipment (apparently quite unnecessarily).

'Syrena' was in fact an experimental capsule, used in conjunction with the Soviet 'splav' furnace, to produce highly homogeneous tellurium cadmium and telkurium/mercury alloys. Such alloys, of substances with very different molecular weights, are of considerable interest in semiconductor technology, and, in fact, both the Czech and East German cosmonauts carried out analogous experiments. It appears from Hermaszewski's description, however, that although the Soviets provide the furnace, the various Comecon nationalities are responsible for the contents of the individual capsules and for working out the results—as well as for the symbolic code-name under which the experiment is performed.

This led, naturally, to the whole question of Comecon participation in manned space-flight. Hermaszewski stressed that, although he only knew the Czech and East German cosmonauts well—the other future crew-members arrived only towards the end of his own training—he felt confident that all the countries concerned (even Mongolia and Cuba) would have their own "concepts" to contribute to the programme. "But obviously that's their secret for the moment." He stressed, moreover, that there are a number of joint experiments in the programme, for example, some of the photographic surveys, and such things as the joint Polish-Soviet experiment on heat level.

As for Poland's future plans in space, he was noncommittal. It was beyond his competence, he said, to comment on the future of the *Interkosmos* manned programme, when the present series of joint flights is complete, or on the possibility of wider international cooperation in space, perhaps on a UN basis. He stressed, however, that "We are only at the beginning of the Polish space programme."

Fields of special interest for the future included: "cosmic technology ('Syrena' was an example of this); space physics; and experiments with some immediate effect on our economy — telecommunications, medicine, biology and so on".

As for his own future, Hermaszewski said that he is now doing a great deal of work with young people, including a special TV programme. "I have had a great many letters from the young people of our country, and they are all very proud that Poland has begun the peaceful exploration of space!" ☐

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US slashes funds to WHO and other UN agencies

In a pre-recess move that caught administration officials off-guard, the US Congress has made reductions totalling over $27 million in next year's contribution to a number of key specialist agencies of the United Nations, including the World Health Organisation (WHO), the Food and Agricultural Organisation (FAO), and the United Nations Educational, Scientific and Cultural Organisation (UNESCO).

Furthermore the Congress has decreed that none of the remaining $200 million contributions to these agencies and the UN itself may be used for the purpose of "technical assistance". The agencies are now trying to work out whether they can accept contributions under such conditions; if not, then 25% of the budget of each which is currently provided by the US, has been placed in jeopardy.

Hardest hit has been WHO, to which the US contribution has been reduced by 43%, or $20.9 million; if this cut remains standing, the net result will be a 10% reduction in the organisation's total budget. Other cuts are: FAO reduced by $3.2 million (10.7% of the US contribution); UNESCO by $625,000 (2.1% of the contribution); World Intellectual Property Organisation by $110,000 (100% of contribution); and the UN itself by $2.85 million (2.5% of total contribution).

The decision to cut the contributions resulted from floor amendments accepted during debates in both the House and the Senate on the budget of the State Department, from which such payments are made. President Carter was unwilling to veto the whole bill, but while signing it into law he said that the amendment "compromises this government's ability to fulfil its legally binding financial obligations".

The President added that he intended to recommend the prompt restoration of the technical assistance funds and eliminate the restrictive language. Administration officials said last week that it was proposed to do this with a supplementary appropriations bill that will be presented as soon as the new Congress meets in January. However, even if the language is changed, it is unlikely that the money will be made available in time to meet the next payments to the agencies, due in February.

The cuts reflect growing concern by some of the more conservative members of Congress over contributions made by the US to international organisations over which they have minimal control; unlike "voluntary" contributions to institutions such as the World Bank, the size of which is related to voting power in the institution, the specialised UN agencies, to which contributions are assessed broadly on grounds of the ability to pay, operate on a one-country-one-vote principle.

"Voluntary" contributions come under the foreign aid bill; and this year Congress has attached to the bill conditions that prohibit its money being spent in countries such as Vietnam and Cuba of whose political regime it disapproves. "Assessed" contributions, however, although paid for out of the State Departments budget, are determined by international agreement, they are not supposed to be subject to the same type of pressures.

In previous years, attempts by members of the Senate to delete the technical assistance money from the department's appropriations and to transfer it to the foreign aid bill have been successfully resisted by the House. This year, however, caught up in the pre-recess rush of legislation, the House voted by 191 to 143 not to reject an amendment added by Senator Jesse Helms, whose cuts and restrictions subsequently passed into law.

"The use of assessed contributions to fund technical assistance is a device to tax the developed nations for the benefit of the undeveloped nations without proper consent from the developed nations", Representative John H. Rousselot, a former official of the right-wing John Birch Society, told the House in supporting the Helms amendment.

"It is changing the very nature of UN agencies away from programmes beneficial to all member nations and towards programmes of special interest to particular areas of the world."

David Dickson

Denmark expands research on Ante-natal diagnosis

The Danish government has taken a significant step towards including ante-natal genetic diagnosis in the general health service. It has approved a plan for expanding laboratories and research centres involved in ante-natal diagnosis and last month granted funds for making it available to all 'risk patients' within approximately five years.

In 1972 the Danish Medical Research Council (MRC) decided to support and coordinate the work of several laboratories on congenital deformities and metabolic diseases. In 1975 the Ministry of the Interior (MI) appointed a committee to investigate the extent to which ante-natal diagnosis should be developed in Denmark, to outline how it should be developed and, finally, to investigate its economics.

The MI-committee presented its report (MI-report 803/1977) in February 1977. It concluded that the community would gain considerably in both human and economic terms if ante-natal diagnosis was given high priority in the general health service.

The report considered chromosomal diseases and inbred metabolic disorders. The MI-committee estimated that about 4,000 chromosomal analyses would be needed yearly to check all risk groups —pregnant women older than 35 and those who have previously given birth to a child with a chromosomal disease. It recommended that seven centres specialising in aminocentesis—which is necessary for chromosomal analysis—be established. At present about 1,000 such analyses are done yearly.

Inbred metabolic disorders are very rare and diagnosis of each illness within this broad category requires its own special technique. Nevertheless, the report mentions several independent calculations which have found that for several specific diseases the benefit of ante-natal diagnosis and abortion in the case of positive results outweighs the cost. The main difficulty, however, lies with identifying those at risk. Either the whole population or selected groups can be screened. At present the Danish population as a whole is screened for phenylketonurea. The MI-committee has recommended that population screening is now extended to include congenital myxodoema.

Any further expansion of population screening for metabolic disorders must await the results of additional research. The MI-committee recommends, however, that programmes for selective screening should be incorporated into a few genetic research centres now. Centres should be established in Copenhagen and Aarhus, it says. They should coordinate all ante-natal biochemical diagnosis in Denmark. Six million kroner should be enough for about 2,000 such analyses a year—the current estimate of the need.

A communication from the National Health Service to all Danish hospitals, medical doctors and midwives marked the initiation of the new programme on 1 October. All pregnant women in Denmark belonging to a risk group are now offered genetic counselling and diagnosis. At the same time the financing of most prenatal genetic diagnosis will pass from the MRC to the general health service.

Sven Erik Godtfredsen

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news in brief

● **Explosion hazard at Windscale:** An unexpected and potentially dangerous build-up of hydrogen in one of the storage silos at the Windscale reprocessing plant has forced British Nuclear Fuels Ltd to close the plant temporarily. The build-up of hydrogen was discovered on Tuesday, 3 October. Safety procedures were instigated to bring it quickly under control.

Hydrogen is produced by a reaction between the magnox cladding material that is stripped from irradiated fuel elements before they go into the reprocessing plant, and the water in which they are stored in the silos. The hydrogen build-up was caused by a sudden increase in the temperature in the silos, due to the settlement of the magnox; this speeded up the reaction.

The temperature was lowered when the stand-by cooling system was switched on. The hydrogen was dispersed by forced air ventilation of the silos, but this in turn caused a discharge of radioactivity into the area around the silo. Although the discharge was within the safety limits, people working in the area had to be evacuated and decanning (pictured above) and reprocessing procedures halted.

● **Austria decides against nuclear energy:** Austria's new nuclear power station at Zwentendorf near Vienna may never be used, even though it is already loaded with the necessary radioactive elements. This follows a referendum on nuclear energy in which the Austrian people voted by a small majority against the use of nuclear energy in their country. In the poll, 50.5% of the votes were opposed to nuclear energy, and 49.5% in favour.

The referendum puts the Austrian Chancellor, Dr Bruno Kreisky, in a difficult position. The Zwentendorf power station forms a major part of the government's energy policy, and the ruling Socialist Party now has to decide what to do with it. It could be converted to a conventional power station. Dr Kreisky, who has described the referendum result as "a personal defeat", has however announced that he will not carry out his threat to resign if the vote went against atomic energy.

● **"No scientific barriers to nuclear waste disposal" says US report:** Despite limitations, existing scientific knowledge and technology is adequate to proceed with selecting and characterising sites for the permanent disposal of radioactive waste, according to a report issued last week by the US Department of Energy.

The report says that the successful isolation of radioactive waste from the biosphere "appears feasible for periods of thousands of years" provided that a systems approach for selecting the geologic environment, repository site and waste-form is utilised rigorously.

And it says that a broad range of underground media, such as salt, basalt, granite and shales, should be considered to provide a "conceptual framework" for program planning and establishing near-term priorities.

The report was prepared by an inter-agency review group chaired by Dr John Deutch, director of the office of energy research. After 30 days for public comment, a revised version will be prepared for the President as the basis for an administration policy on the handling, transportation and storage of nuclear wastes.

It recommends that an executive planning council be set up as part of a partnership between the federal government and individual states to plan and implement the nuclear waste programme. And in order to assist in the progression to high level repositories, the report suggests that one or more intermediate facilities be established in several emplacement media and geologic environments "at the earliest practicable date." If an alternative to salt is selected for the first high-level waste depository, this is unlikely to be ready until 1992 at the earliest, the report says.

● **US and China agree to exchange research workers:** China is expected to send over 500 postgraduate students and research workers to the US over the next year under the terms of a verbal agreement made in Washington last week between the National Science Foundation and a visiting delegation from the People's Republic of China.

The most popular subjects are likely to be physical and biomedical sciences, engineering and applied technology. In exchange about 60 US researchers will visit China, although primarily to study subjects in the social sciences, language and literature, and archaeology and art.

In addition to these exchanges, many more Chinese students will come to take courses in the US through private arrangements with US universities; the first of these have already started courses at Stanford University and the University of California at Berkeley.

Under the terms of the agreement, which was reached after a two-week visit by the delegation which had included 14 universities and colleges, each side agreed to use "its best efforts" to fulfil requests for study opportunities, and to pay the costs for its participants.

● **Lund looks at UNCSTD:** How are preparations for next year's United Nations Conference on Science and Technology for Development (UNCSTD)? "Rather bad but not disastrous", says Docent Jon Sigurdson, director of the University of Lund's Research Policy Program and co-editor of the Lund Letter, circulated regularly to governments and people interested in UNCSTD. "There are organisational problems, and if the organisation doesn't work well we can't pinpoint the questions that should be discussed."

It is well known amongst officials working with UNCSTD matters that the UNCSTD Secretariat and the UN Office for Science and Technology have found cooperation difficult, with each one trying to make the conference its own show. Similar problems have arisen between the Secretariat and various UN agencies—UNESCO, for example—and UNCSTD's Secretary-General Da Costa, who has been on sick leave for extended periods, has not been able to provide the strong leadership necessary to bring the squabblers into line.

"The first phase of the preparations—the national analyses leading up to the national papers—is nearly over now", says Mr Lennart Båge of the Ministry of Foreign Affairs. "We are now anxiously waiting for the Secretariat to analyse these papers. As the third preparatory committee meeting has been postponed until January, we need good background documentation in good time for the meeting to make it an efficient starting-point for the international negotiations leading up to the conference."

The Research Policy Program is itself about to begin going through the sixty or so national papers to hand to see which themes they have taken up and where their deficiencies lie. "From what we have seen", says Docent Sigurdson, "most national papers seem to have dealt only with item one on the conference's agenda: science and technology for development. Very little attention seems to have been paid to the other three items, especially the last on: science and technology for the future".

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news and views

Hormones and cell culture

from Philip S. Rudland

It is rare in science these days for a conference to be convened in memory of any one person, but such was the high regard in which Gordon Tomkins was held that the Sixth Cold Spring Harbor Meeting on Cell Proliferation* was convened in his memory in a field in which he was one of the father figures, that of Hormones and Cell Culture. Gordon Tomkins was one of the first to consider that animal cells in culture could be used almost like bacteria for following molecular events, and in particular the effects of hormones on various cell functions. Nowadays the Hormones and Cell Culture field seems to be split into two camps, one concerned with the isolation of growth factors and their mode of action on cell proliferation and the other with the action of hormones in inducing cellular differentiation.

Cell proliferation

On the cell proliferation side R. Iglesias (Santiago) emphasised the historical importance of hormones, particularly oestrogens, in inducing tumours in animals, although it is uncertain whether oestrogen usually affects cell proliferation directly as reported for a human breast cancer cell line (M. Lippman *et al.* National Cancer Institute, Bethesda), or through intermediary growth factors (estromedins) proposed and identified by D. Sirbasku *et al.* (University of Texas, Houston) as necessary for the growth of cells from some oestrogen dependent tumours *in vivo*. The major mitogens for cultured cells, the so-called growth factors, seem to be becoming purer and the purification of one from platelets was extensively discussed (R. Ross *et al.* University of Washington, Seattle; C. Heldin *et al.* Institute of Medical and Physiological Chemistry, Uppsala). A new growth factor of less than 3,000 daltons has been isolated from bovine pituitaries; this factor specifically stimulates the growth of a rat mammary epithelial cell line from a hormone-dependent transplantable rat mammary carcinoma (T. Kano-Sueoka *et al.* University of Colorado, Boulder). Vasopressin could act like a growth factor in 3T3 fibroblastic cells (H. Rozengurt, Imperial Cancer Research Fund, London). The importance of hormones and growth factors in promoting cell proliferation was emphasised when certain of these agents were reported to support the growth of several different cell lines in the absence of serum (J. Bottenstein *et al.* University of Calif. San Diego; Serrero *et al.* SPNCI, Nice; Lippman *et al.*). Cultured cells and tumours are also proving a rich source of growth factors. The discovery of growth factors was stimulated to some extent by the hypothesis that tumour viruses produced their transforming action on cells, at least in part, by the production of endogenous polypeptide growth stimulatory factors (G. Todaro, National Cancer Institute, Bethesda). Todaro now reported that a human fibrosarcoma released growth-polypeptides which could interact with receptors which normally bind a serum growth factor, MSA. Conversely however, normal rat or hamster liver released growth-promoting activity for virally, spontaneously and chemically transformed cells but which did not stimulate their normal counterparts (A. Lipton *et al.*, Hershey Medical Centre, Hershey).

Before discussing the action of growth factors further one needs to know how and where they act in the cell cycle. A. Pardee (Sidney Farber Cancer Institute, Boston) compared two different classes of model for the cell cycle which could account for the variability in the distribution of cellular intermitotic times, deterministic Gaussian types and random or probabilistic types. He suggested that a deterministic model giving a reciprocal normal distribution could be made to fit the daughter cell generation times as well as the semilogarithmic or first order plots of the probabilistic model, in contrast to a recent paper (Shields *Nature* **273**, 755; 1978) which claimed the contrary. However it seems that the choice between the two types of model at least is deterministic, depending on which side of the Atlantic the decision is made. Two slightly different versions of how growth factors affect the cell cycle were presented. First C. D. Stiles *et al.* (Sidney Farber Cancer Institute, Boston) considered that one growth factor, platelet factor, 'committed' quiescent 3T3 fibroblastic cells to a process which eventually led to a first order increase in the rate of cellular entry into S phase, while platelet-poor plasma was required to allow the cells to 'progress' to the increased rates of DNA synthesis. However L. Jimenez de Asua *et al.* (Friedrich Miescher-Institut, Basel) considered that the growth factors fibroblast growth factor and prostaglandin $F_{2\alpha}$ delivered both signals, one to commit the cells to change their growth rates and the second to increase the rate of entry into S. Hormones such as insulin and glucocorticoids alter only the second response.

Where do growth factors act?

The biochemical as opposed to the kinetic mode of action of growth factors is also unclear. Two models have arisen; the earlier envisaged that growth factors bind to cell surface receptors and then trigger intracellular 'messengers' without entering the cell; the more recent model considers that the growth factors are 'internalised' and go to 'control sites' inside the cell. Support for the earlier model was provided by the carefully controlled experiments of D. Cunningham *et al.* (University of California, Irvine) with the protease thrombin. He has found that this can still stimulate chick embryo cells' division even when attached to Sepharose beads which cannot enter the cell, although unattached thrombin was internalised (J. Quigley

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et al., State University of New York). A series of speakers starting with S. Cohen (Vanderbilt University, Nashville) showed that epidermal growth factor (EGF) could be internalised and eventually degraded in lysosomal vesicles. Whether internalisation of EGF is required for mitogenic activity, however, is unknown, although C. Fox (University of California, Los Angeles) found that the concentration of EGF required for half-maximal binding to the surface receptor was much less than for internalisation. The latter corresponded more closely to the concentration required to stimulate maximum rates of DNA synthesis, which suggested that internalisation might be the limiting step. Internalisation was not a unique property of EGF, however, since thyroid-releasing-hormone (A. Tixier-Vidal *et al.* Collège de France, Paris) and melanocyte stimulating hormone (A. Lerner *et al.* Yale University) are also internalised. If the first model for the action of growth factors is correct, there is a requirement for second messengers or a chain of messengers to continue the 'growth stimulus' inside the cell. A much simpler example of such a system is that of insulin control of a single enzyme, glycogen synthetase, in rat adipocytes (J. Larner *et al.* University of Virginia, Charlottesville). Two separate biochemical mechanisms were proposed to control a multiply-phosphorylated enzyme, glycogen synthetase. Phosphorylation of this enzyme and hence its activity were then controlled by a cyclic AMP-dependent protein kinase, a cyclic AMP-independent kinase and by the calcium-stimulated action of phosphorylase *b* kinase. Models of the first type for the action of growth factors may then involve biochemical pathways of nightmarish complexity.

Differentiation

Studies of the effects of hormones on differentiation processes emphasised the reciprocal nature of proliferation and differentiation. Thus in cultured adrenocortical cells, fibroblast growth factor inhibited steroidogenesis, the differentiation response, while ACTH, which stimulated steroidogenesis; inhibited cell proliferation (G. Gill *et al.* University of California, San Diego). The evidence that ACTH acts through cyclic AMP in this case was good since Y-1 adrenal cells could be selected which were deficient in some component of the adenylate cyclase in cyclic AMP-dependent protein kinase activities and these also failed to respond to ACTH (B. Schimmer *et al.* University of Toronto). Different inducing agents could also commit more primitive tissue culture cells (stem cells) to a new cell type. Thus vitamin A induced the formation of endodermal cells from mouse embryonal carcinoma cells *in vitro* (S. Strickland, Rockefeller University, New York), and dimethylsulphoxide (P. S. Rudland *et al.* Imperial Cancer Research Fund, London) and other agents (J. Lever, Salk Institute, San Diego) which induced Friend erythroleukaemic cells to synthesise globin, caused a rat mammary carcinoma stem cell line to differentiate into alveolar-like secretory cells. Interestingly the effect of hormones on the responsive cell in the animal may not necessarily be direct, but through other cell types. Thus during embryonic development the mammary rudiment of the male mouse regresses as a result of androgen action, not on the epithelium, but on the surrounding mesenchymal cells which in turn causes the epithelium to become necrotic (K. Kratochwil *et al.* Osterreichische Akademie der Wissenschaften, Salzburg).

The majority of the work on hormonal control of differentiation at the molecular level was concerned with the modulation of amounts of intracellular enzymes and secreted hormones in cells which were already differentiated, rather than changing the cell to a new type as above. Hormones mainly exert their effect on specific gene expression by increasing the amount of specific cytoplasmic mRNAs. Moreover in spontaneous rat hepatoma cell variants the control of the production of more than one gene product occurs at different, unconnected steps beyond the interaction of the glucocorticoid with its cytoplasmic receptor (E. Thompson *et al.* National Cancer Institute, Bethesda). In a *tour de force* of molecular biology J. Baxter *et al.* (University of California, San Francisco) first produced a working hypothesis that glucocorticoids rapidly modify chromatin structure (as measured by the binding of *E. coli* polymerase) in a gross way that nevertheless affects the expression of only a few genes in rat pituitary (GC, GH$_3$) cells, notably the growth hormone gene. He then reported the nucleotide sequences of the DNA complementary to human and rat growth hormone and human placental lactogen mRNAs, which were remarkably similar, and finally showed that a new protein containing immunoreactive growth hormone sequences could be obtained in bacteria by ligating cloned rat growth hormone cDNA and the ampicillin resistance gene of another plasmid. Thus the conference had come full circle from Gordon Tomkin's original belief in that a hormonally-regulatable gene from cultured animal cells could now be expressed in a bacterium. The conference ended with a highly enjoyable jazz session, a fitting tribute to Gordon Tomkins, the jazz musician. □

Antenatal diagnosis of genetic defects

from Bob Williamson

A NEW and most exciting method of antenatal diagnosis is reported in the *Lancet* (**ii**, 910, 1978) by Y. W. Kan and Andree Dozy, of the San Francisco General Hospital. They have applied recombinant DNA techniques developed to study the organisation of the human globin gene family, to diagnose successfully a fetus at risk for sickle cell anaemia. The method is very sensitive and safe and will no doubt be applied immediately in similar cases. However, the implications are far wider, for this result promises that any diseases for which a gene-specific probe can be identified may well be diagnosed in the same way.

As readers of these columns know, the arrangement of genes for which specific probes are available can be studied by restriction enzyme analysis to provide a detailed physical map of the coding and surrounding sequences. The human β globin gene occurs only once in the haploid genome, is separated from the related gene coding for the globin chain by some 7,000 base pairs, and contains at least one intervening sequence several hundred base pairs long. The map of restriction sites round the gene has been constructed by several groups, and there is broad agreement on its appearance (see *News and Views* **276,** 13; 1978). Kan has now shown that for people of white, black or Asian origin with no haematological disorder, there are usually sites for the restriction enzyme *Hpa*I generating a fragment 7.6 kb (kb=1000 bases) long and including the β globin gene. This was always the case for the whites and Asians tested. Blacks with sickle-cell anaemia have almost invariably lost the 3′ *Hpa* site, and a new one, which gives rise to a fragment 13 kb long is found instead. (Another restriction site giving a 7.0 kb β globin gene fragment is also occasionally found but this seems to be a polymorphism among blacks unrelated to sickle-cell anaemia). Natur-

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ally, in general the cases with homozygous sickle-cell disease had a 'double dose' of the 13.0 kb fragment while heterozygous carriers had one 13 kb fragment and either the 7.0 or 7.6 kb fragment.

Sickle cell anaemia is due to a single base change in the DNA causing a substitution of valine for glutamic acid in position 6 of the β-globin protein sequence; this change presumably has nothing to do with events thousands of base pairs away. What seems to have happened is that the sickle cell mutation, which confers resistance to malaria in heterozygotes, just happened to occur in an individual who had either lost the site for *Hpa*I at 7.6 kb, or whose genes had undergone some rearrangement at that site. Then the rearrangement or change far away from the globin gene was 'fixed', to be locked in linkage to it in subsequent generations.

The fact that a second linked polymorphism has been found (and several have been suggested for the other globin genes as well) argues that these are rare but by no means unknown events. They may not occur often at any given site, but there is a likelihood (if one can generalise, as usual, from the globin genes to other structural genes) that several could be identified within a few thousand base pairs of any gene. Any mutation thus carries with it a characteristic detritus of surrounding gene material, in arrangement and sequence. This in turn implies that an advantageous mutation becomes 'fixed' more rapidly than random mutational drift occurs in unselected sequences.

One of the most remarkable aspects of Kan and Dozy's result is the sensitivity of the method. Only 15 ml of amniotic fluid were taken. There was sufficient DNA in the foetal cells suspended in this sample of fluid to allow direct analysis without further cell culture. DNA from the parents' white blood cells, and from any siblings, must also be prepared, to ensure that the linkage is valid in the case under study. Hybridisation and autoradiography take only a week, allowing a result to be obtained earlier than with methods that require a foetal blood sample, which must be taken later in the pregnancy. Termination can be offered in good time, and the investigation has none of the technical risks associated with taking a foetal blood sample, and does not require such sophisticated equipment.

By recombinant DNA techniques, the entire human genome can be easily cloned in lambda phage or in plasmids; at least four groups have done so already. These 'clone libraries' are being made available to groups wishing to study particular diseases and who have developed gene-specific probes. Consider, for example, identifying a similar linkage group for a condition such as haemophilia. Polysomes making factor VIII protein could be precipitated with antibodies, the RNA isolated and a DNA complementary copy cloned in a plasmid. This can be used to hunt for the gene sequence, and polymorphisms identified–attempts would be made to link these to the haemophilia mutation. If this can be done (and for a recessive illness, there are good theoretical reasons to think that it can) there is no need to look at the very small sequence change, often involving a single base pair, causing the disease—linkage is enough for antenatal diagnosis. Another advantage of the method is the ease of diagnosing heterozygotes—they will have one, but not both, genes affected—a pattern easily seen using restriction enzymes and hybridisation.

Several diseases will be candidates for this approach in the months ahead. Some cases of β-thalassaemia do not involve a gene deletion, but could be linked in a similar way to specific restriction sites, particularly where family studies are possible. It should be easy to develop gene-specific probes for single gene defects where the affected protein is known, such as agammaglobulinaemia, Tay-Sachs disease and Lesch-Nyhan syndrome. Even conditions where the protein affected is not yet identified, such as Duchenne muscular dystrophy, might be studied using recombinant DNA in this way.

Since it is often thought that this technology increases the overall abortion rate by making more knowledge available to parents, perhaps it should be noted that Kan and Dozy's case turned out to have the heterozygous sickle cell trait was not terminated, and will grow up haematologically normal. Far from leading to more terminations, the availability of accurate antenatal diagnosis for severe hereditary disease should allow a decrease in abortion, since parents can continue a pregnancy knowing that a child will be healthy rather than terminate all pregnancies for fear of a possible severe illness and death. □

Positrons at the centre of the Galaxy

from J. J. Quenby

AFTER a long struggle, balloon experiments seem to have established the presence of detectable γ-radiation due to positive electrons at the centre of our Galaxy. Whether this radiation is ultimately telling us about the remains of supernova explosions, the cosmic ray distribution, the physics deep inside the magnetic fields of pulsars or black holes remains a largely open question.

Positrons can react either in flight or at rest with electrons, bringing about their mutual annihilation and the production of two gamma rays at an energy of 511 keV. If the annihilation is at rest, a sharp 'line' flux of photons results but if the interaction is in flight, Doppler broadening of the feature spreads out the apparent frequency of photon emission. A sharp line is the characteristic signature sought by balloon-borne detectors used in γ-ray astrophysics. Even though the positrons responsible may be very slow, it is not necessary for all the annihilation radiation to produce a line. At low matter densities, normal in astrophysical situations, positrons and negative electrons may form a bound state known as positronium. 75% of all annihilations may occur in a 3-photon decay of this state, producing a wide spread of photon energies.

The earliest indication of line radiation from the galactic centre was obtained by Johnson, Harden and Haymes (*Astrophys. J.* **172,** L1; 1972) using a crystal scintillator with a resolution of ±24 keV. This group later improved their technique getting a resolution of ±11 keV (Haymes *et al. Astrophys. J.* **201,** 593; 1975). Both of these results gave line positions more than one standard deviation away from the 511 keV energy. A more definitive study has now been reported by Leventhal, MacCallum and Stang of Bell and Sandia laboratories (*Astrophys. J. Lett* **225,** L11; 1978) using a rather difficult technique involving cooled germanium detectors. A resolution down to 3 keV is achieved by keeping the solid state detector at around 100 K or less. One problem encountered by these workers was the instrumental background line at 511 keV due to cosmic ray interactions. However, by pointing on and off the source, a galactic centre 511 keV line flux of 1.2×10^{-3} ph S^{-1} cm^{-2} was found, together with some probable spreading due to the additional 3-photon decay mode. Against this confirmation of the Haymes group's work is a statement from the satellite experimenters on HEAO-1 that as yet no line flux around 500 keV is seen (HEAO-1 Newsletter 2, April 1978). Here again only a crystal detector with broad band energy resolution was used.

What explanation can be advanced

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for the striking Leventhal *et al.* result? Positrons may occur deep within the magnetic field regions of pulsars, close to the surface, due to energetic proton reactions or indirectly from electric field acceleration of electrons. However, the strong gravitational field might be expected to significantly shift the line frequency from the 511 keV level.

For a long time it has been noted that radioactive decay products arising from nuclear burning at the time of supernova explosions may produce observable γ-ray lines (for example, Colgate, *Astrophys. Space Sci.* **8**, 457; 1970). The explosion is preceded by a silicon burning process in which reactions with free α-particles leads to the synthesis of elements near iron in the periodic table. In particular the decay of the ^{56}Co and the ^{44}Sc produced includes long half-line positron emission. Some fraction of the exploding star enters the interstellar medium. If the positrons come from this blown-off envelope, spatial and time variations are expected in the line flux.

Another approach to the explanation is to consider the interactions with the residual medium experienced by energetic cosmic ray particles in the Galaxy. In particular, particles above 10 MeV can excite positron emission from C, N and O atoms. Ramaty, Stecker and Misra (*J. geophys. Res.* **75**, 114; 1970) have made detailed estimates based on this mechanism but demand a cosmic ray energy density more than 10 times that seen near Earth. One way out of this problem is to realise that explanation of the 50 MeV γ-ray flux from near the galactic centre first seen by OSO-III (Kraushaar *et al.*, *Ap. J.* **177**, 341; 1972) may require a similar cosmic ray density enhancement.

Further information on the line emission should come when HEAO-C is launched, carrying a cooled germanium X-ray detector and also in the more distant future, from a larger version of this apparatus on board the GRO mission. It is then that the theoretical possibilities may be sorted out and other more speculative sources of positrons such as evaporating primordial black holes may perhaps be brought into consideration. □

Channels in membranes

from Venetia A. Saunders

THE latest applications of model membrane systems to the study of bacterial membrane function has provided an important insight into the role of the major outer membrane protein—matrix protein—and the mechanism underlying the action of the bactericidal colicin K protein.

The outer membrane of Gram-negative bacteria such as *Escherichia coli* is freely permeable to low molecular weight hydrophilic molecules such as glucose and amino acids (for a recent review see Di Rienzo, Nakamura & Inouye *A. Rev. Biochem.* **47**, 481; 1978). Recent results indicate that several outer membrane proteins have a role in the permeation of such molecules through the membrane.

Nakae (*Biochem. biophys. Res. Commun.* **71**, 877; 1976) has shown that incorporation of matrix protein (which corresponds to porin, molecular weight 36,500), a major outer membrane protein from *Escherichia coli*, into artificial membrane vesicles restores their permeability to a variety of saccharides. Such reconstituted vesicles show similar molecular sieving properties to those of the intact cell membrane. (Estimates of exclusion limits range from about 600 to 900 daltons for saccharides). It has been tentatively proposed that one function of matrix protein is to generate aqueous transmembrane channels that permit the rapid diffusion of small hydrophilic molecules. This notion is supported by the finding that mutants of *E. coli* lacking matrix protein are defective in passive diffusion (Bavoil & Nikaido *Molec. gen. Genet.* **158**, 23; 1977). There is also evidence (Schnaitman, Smith & de Salsas *J. Virol.* **15**, 1121; 1975) suggesting that matrix protein can be utilised as a receptor for the temperate coliphage PA-2 and that the amount of this protein is substantially reduced in lysogenised strains of *E. coli*. This could provide a mechanism for the exclusion of superinfecting phages. Ultrastructural studies of Steven and coworkers (*J. Cell Biol.* **72**, 292; 1977) have revealed that the matrix protein is present in a highly organised array within the architecture of the outer membrane: 10^5 copies of polypeptide per cell are arranged in a periodic monolayer which exhibits three-fold symmetry and triplet indentations (possibly corresponding to channels).

A recent paper from Schindler and Rosenbusch (*Proc. natn. Acad. Sci. U.S.A.* **75**, 3751; 1978) now describes the successful integration of matrix protein into planar lipid bilayers and shows that the incorporated protein creates voltage-controlled channels across these membranes. Those channels conduct ions and are freely permeable to small hydrophilic molecules. Analysis of the electrical properties of the membranes indicates that channels are induced irreversibly by voltage and that the number of channels is directly related to the matrix protein content of the membranes. Channels are proposed to be of uniform size, approximately 1 nm in diameter and to assume either an open (active) or closed (inactive) state. Schindler and Rosenbusch speculate that the closing of channels, which is favoured by high potentials, could be a mechanism for regulating permeability of the outer membrane. It is interesting to note that channel induction in the bilayers is voltage-dependent between *p*H 6 and *p*H 8, but is spontaneous at *p*H 10. The fraction of active open channels in bilayers at *p*H 10 is roughly equivalent to that in the bacterial cell, whereas the fraction of open channels in bilayers at physiological *p*H is significantly less. This discrepancy presumably reflects the inevitable artificiality of *in vitro* membrane systems.

In this issue of *Nature* (page 159) Schein, Kagan and Finkelstein report the use of planar phospholipid bilayer membranes to identify the precise mechanism of colicin action. They find that treatment of planar phospholipid membranes with colicin K also results in the formation of voltage-dependent ion-permeable channels, with the number of channels generated dependent on colicin concentration. These observations are consistent with the known effects of the E1 functional group of colicins (K, E1 and Ia) on the bacterial cell. They exert their bacteriocidal effects by a discharge of the 'energised state' of the cytoplasmic membrane and promotion of a net efflux of ions from the cell. One point of interest is that preparations of colicin K from *Proteus mirabilis* and *E. coli*, which are apparently specified by identical plasmids, differ somewhat in respect of their molecular weights and their quantitative effects on the artificial membrane system.

Schein *et al.* suggest that a single colicin channel could deplete a bacterial cell of all its potassium and other small ions within minutes. The relatively indiscriminate flux of ions through the channels could provoke at least partial depolarisation of the membrane. Since the energy to drive active transport systems derives from the transmembrane potential and the *p*H gradient (in accord with the chemiosmotic hypothesis), membrane depolarisation could in turn disrupt active transport. Indeed it is possible that depolarisation *per se* could lead to channel closure. The finding that E1 type colicins create voltage-dependent channels in artificial

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Will detente kill millikelvin research?

from P. V. E. McClintock

RECENT exciting advances in physics at millikelvin temperatures have been made possible by an ample and relatively cheap supply of helium-3 for operating dilution refrigerators, but it looks very much as though this happy situation is now coming to an end, with an impending world-wide shortage of the gas. The Radiochemical Centre at Amersham, for example, is not accepting any further orders: even when the present backlog of orders, some dating back to the beginning of the year, has finally been cleared, it is apparently by no means certain that further supplies will become available for sale.

One of the principal uses of helium-3 is as the working fluid of the dilution refrigerators which have become the work-horses of millikelvin research. The principle on which they operate is extremely simple, just depending on the cooling effect which takes place when liquid helium-3 is allowed to dissolve in liquid helium-4. The refrigerator is usually arranged so that the process is continuous, with provision being made for separating the two isotopes again in a still, and for recirculating the ^{3}He back to the mixing chamber through a series of heat exchangers in which it is cooled by the cold dilute mixture leaving the chamber. Optimising the design of such machines is quite a complicated exercise, but progress over the past few years has been rapid: the refrigerators developed in Grenoble by Frossati, for example, are now able to sustain temperatures of around 2.5 mK in continuous operation, which is lower by a factor of four than the best available commercial machines of only a couple of years ago.

The present helium-3 famine arises, probably, for three main reasons. First, there has been a decided increase in the number of research groups building or buying dilution refrigerators, partly stimulated by the discovery of superfluidity in liquid helium-3 itself: all these machines require an initial charge of helium-3. Second, the relatively large machines which people are now tending to build require correspondingly large quantities of helium-3; this is particularly true of the Frossati designs, which incorporate exceptionally large diameter tubes within and between the lower heat-exchangers in order to reduce viscous heating effects as the liquid moves through the system. Finally, there is detente.

The ample supply of helium-3 enjoyed by cryophysicists in recent years has been produced as a byproduct of the nuclear weapons programme. The tritium used in thermonuclear devices decays through β-emission to helium-3, with a half-life of about 12 years. Although nobody seems very keen on discussing the exact arrangements made in practice for collecting the helium-3, one may infer that it is very much less convenient to do so once the weapon is in its final form ready for use, so that the ready availability of gas for sale to researchers and the cryogenics industry probably depends on continued manufacture.

Although the dilution refrigerator in its present form can certainly be regarded as a byproduct of the thermonuclear arms race, and any slackening of the latter must be warmly welcomed, it will be sad indeed if the excitingly rapid progress now being made in the millikelvin field is seriously curtailed. There are, however, two reasons to remain fairly optimistic about the long term outcome.

First, one may note that after being set up with its initial charge of helium-3, very little of the gas need get lost from a dilution refrigerator, such losses as there are occurring only by leakage or through human error, both of which can be minimised by careful design. So provided that there is a slowdown in the rate at which new machines are being commissioned, the present gap between demand and supply may eventually decrease again. Unfortunately there is no sign of this happening just at the moment.

Second, there always remains the possibility of extraction from natural helium-4, which usually contains a few parts in 10^7 of the light isotope. This certainly can be accomplished—at a price—and, indeed, was used as a small scale method of helium-3 production before the advent of extensive thermonuclear weapons programmes. There is a considerable variation in the helium-3 content of the gas from different wells. By choosing the right well and using modern isotopic separation techniques it just might be possible to produce helium-3 at a price not too much above the present £75 per S.T.P. litre. Furthermore, if the rumours about certain Polish gas wells prove to be correct—that they produce helium containing about 1 part in 10^5 of helium-3—a return to extraction from the natural helium could even lead to a reduction in the current price, at the same time making Poland the World's chief supplier of helium-3.

Until an alternative source of helium-3 is developed, detente is undoubtedly going to bring a measure of deprivation to the millikelvinists.

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membranes is likely to provide a novel approach to the exploration of membrane function. ☐

Complexity and stability

from N. MacDonald

THE relationship between the complexity and the stability of an ecosystem has been debated for some years by mathematically inclined ecologists, and has occasioned the choice of title for an extremely influential book (May, *Stability and Complexity in Model Ecosystems*, Princeton, 1973). A possible relationship is indicated by a simple argument concerning a food chain, in which species A depends solely on species B, species B solely on species C, and so on. Any break in the chain eliminates all the previous members A,B . . . On the other hand, if there are alternative paths through a food network, one may expect the general structure of the network to survive the loss of one species. Here complexity is interpreted as the fraction of the pairs of species which directly interact, a quantity which may be termed connectance. Stability is interpreted as survival of all remaining species when one is lost.

As one makes the model more mathematically specific, so one has to be specific in the choice of a measure of complexity, and in the type of stability property that is studied. Some version of connectance, often allowing for an average over interactions of different strengths rather than simply counting the interactions, is usually the measure of complexity, in particular in a series of papers which appeared in *Nature* a few years ago. (Gardiner & Ashby, *Nature* **228**, 784; 1971; May, *ibid* **238**, 413; 1972; Siljak, *ibid* **249**, 280; 1974; Daniels & Mackay, *ibid* **251**, 49; 1974; Roberts, *ibid* **251**, 607; 1974; Gilpin, *ibid* **254**, 137; 1975; Saunders & Bazin, *ibid* **256**,

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120; 1975.) Some criticisms of connectance as a measure of complexity can be found in another paper of this series (Somarjai & Goswami *Nature* **236,** 466; 1972).

From a philosophical point of view one may argue that it is paradoxical to seek a single index to characterise complexity. As argued by R. Rosen (*Int. J. Gen. Systems,* **3,** 227; 1977) the complexity of a system may involve the necessity of employing several parallel descriptions of the system. However connectance does pick out and quantify the intuitively satisfying notion that a collection of N solitary individuals is less complex than a collection of $N/2$ interacting pairs, and this in turn is less complex than a collection in which all the potential $\frac{1}{2}N(N-1)$ interactions take place.

The papers cited interpret stability as the stability of a steady state against small disturbances, that is to say they study the local stability of the equilibrium point of a dynamical system. Working with this narrow concept of stability the earliest of these papers showed that for components interacting at random, high connectance reduces the likelihood of stability. Later papers in the series incorporate restrictions on the interactions that seem obligatory if the system is to be acceptable as a model ecosystem. (For recent views of how this work may relate to observations on real ecosystems, see McNaughton, *Nature* **274,** 251; 1964; Pimm & Lawton, *Nature* **275,** 542; 1978.)

One way of going beyond local stability is by constructing Liapunov functions, which are analogous to the potential energy of a physical system. A recent book (Siljak, *Large Scale Dynamic Systems; Stability and Structure,* North-Holland, 1978) examines how the Liapunov stability of an ecosystem model is affected by the complete or intermittent removal of individual species. The criteria used for stability involve the diagonal dominance of interaction matrices. Thus it is required that individual populations are so stable in isolation that interactions with the other populations cannot destabilise. As the connectance increases, in the sense that interactions between species become stronger relative to the stabilising interactions within species, stability is lost. It is clear that the relevance of this to systems involving predation is remote. Some interaction between the predator species and at least one prey species is essential for survival of the predator. Another possible way of removing the restriction to local stability, with the implied reliance on linearisation of the model, is suggested by a recent paper in a rather different context (Froeschlé, *Phys. Rev.* **A18,** 277; 1978). The topic investigated is the relation between connectance and global behaviour of a certain set of formal transformations. The context of this work is the problem of the transition from integrable (periodic or multiply periodic) orbits to ergodic orbits in Hamiltonian systems, and in systems of formal transformations with area-preserving properties analogous to the Liouville property of Hamiltonian systems. Perhaps the most readable accounts of this problem are some reviews by J. Ford (for example in *Dynamical System Theory for Economists.* (ed. Szego) Springer-Verlag, in press).

Froeschlé takes N (up to 50) two-dimensional area-preserving mappings τ_i of a certain form and defines a multidimensional mapping T for which he can specify that T involves coupling between τ_i and τ_j for $j-i \leqslant N_c$. Thus N_c, which is taken $\leqslant 10$, is the connectance. It turns out that there is a sharp increase, at about $N_c=7$, in the extent to which ergodic orbits predominate. It remains to make a connection between the transition to ergodicity and the onset of instability. In practice the way in which one recognises the emergence of ergodicity is by computing the rate at which two orbits, initially very close, drift apart. This is linear with time for integrable orbits, but exponential for ergodic orbits. When using the local instability of an equilibrium point as the property to be interpreted as system instability, one is concerned with the exponential departure of a slightly disturbed orbit from that point. When one uses ergodicity as the relevant property, one is concerned with the rather more general feature of exponential separation of two initially close orbits. This would seem at least as good a candidate for 'the stability' of the system.

It remains to be seen whether the technical difficulties of investigating ergodicity allow one to work with systems satisfying the rudimentary requirements for a model ecosystem. Hamiltonian systems are well known, as discussed in May's book, to be unsuitable as model ecosystems. However it is now known, as reviewed recently in these columns (*News & Views* **271,** 305; 1978; **274,** 847; 1978) that behaviour analogous to ergodicity can arise in a much wider class of dynamical system. □

Informational suppression in higher organisms

from Peter Piper

MUTATIONS in one gene that correct specific defects in others can be isolated in many organisms and a knowledge of their mechanisms of action can throw considerable light on how various molecular components of the cell interact. A recent symposium on nonsense mutations and nonsense suppressors* reviewed ways in which an important class of these mutations, the so-called 'informational suppressors', partially correct certain defects in the protein-coding regions of structural genes by altering components of the protein synthetic machinery of the cell.

Mutations in the protein-coding regions of structural genes fall into different classes depending on the nature of the mutational change. In the deletion mutants a complete segment of the DNA is absent from the gene and this segment may include part of the protein-coding sequence. In another common class, missense mutants, a base substitution in the coding region of the gene leads to the substitution of one amino acid for another in the protein sequence. This alteration is selected on the basis of a change in the protein's properties, frequently a partial or complete loss of function, and can be corrected by the appropriate tRNA mischarging whereby the aberrent amino acid is replaced by another that restores functionality. Such mischarging can be induced by suppressor mutations in tRNA or aminoacyl-tRNA synthetase genes.

Nonsense mutations produce a base change in the sequence of a gene such that one of the three termination codons of the genetic code, UAG, UAA or UGA, is generated within the protein-coding region resulting in the synthesis of an incomplete polypeptide gene product. They can be reverted by suppressor mutations that allow insertion of an aminoacid in response to the nonsense codon. Similarly, frameshift mutations, which alter the reading frame of an mRNA by base insertions or deletions in the coding sequence can be reverted by suppressors that partially restore the reading frame during translation a short distance after it has been displaced by the frameshift mutation. Although nonsense and frameshift suppressors can be generated by mutation of genes for ribosomal proteins or tRNA modification enzymes, in bacteria and yeast where they have been biochemically characterised most of them arise by mutation of tRNA genes (J. D. Smith, MRC Laboratory of Molecular Biology Cambridge; F. Sherman, Rochester).

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*Held at Aarhus University, Denmark on 9–21. July. Except where specified this article describes communications presented at the meeting.

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More recently it has become possible to search for suppressor mutations in higher organisms by combining developments in the genetics of these organisms with advances in molecular biology that enable the precise nature of many structural gene mutations to be determined. In this way it is possible to show whether unlinked mutations that revert a particular genetic phenotype represent informational suppression or merely the activation of silent genes. Only a limited number of selective systems exist for the isolation of variant clones from mammalian cell cultures and as a result mutant cell lines can only be obtained with mutations at a small number of genetic loci.

One locus that has been extensively studied is that coding for hypoxanthine-guanine phosphoribosyl-transferase (HGPRT). Clones partially or completely deficient in HGPRT can be isolated by selecting cells resistant to certain purine derivatives such as 8-azaguanine (8AG) or 6-thioguanine (6TG). Good evidence exists that mutations in the gene for HGPRT are the basis of the changes in cell phenotype (C. T. Caskey, University of Texas, Austin; M. R. Capecchi, University of Utah, Salt Lake City). From these variants it is possible to isolate revertants that have regained the enzyme activity by selecting cells growing in medium containing hypoxanthine, aminopterin and thymidine (HAT) although this procedure only selects revertants having more than 40% of normal HGPRT activity, as shown by autoradiographic analysis of ^{3}H-hypoxanthine incorporation (J. E. Celis, Aarhus). As such it is not suitable for obtaining informational suppressors of mutations that inactivate the HGPRT gene, since these are unlikely to be able to restore the enzyme activity to such a high percentage of its normal level and not disrupt protein synthesis to a degree lethal to the cell. To be compatible with viability they will probably only restore the enzyme level to less than 20% of its normal value (Celis). The cell lines that can be shown to produce an altered HGPRT are only a small fraction of the variant clones resistant to 8AG and 6TG, and therefore this selection is not very efficient for isolating lines in which to study the process of mutation in animal cells or the structure-function relationship of HGPRT (Caskey).

Until recently it was impossible to verify the nature of the alteration in HGPRT defective clones, and so to classify these as missense, nonsense, frameshift or deletion mutants. However, this problem may soon be overcome. It has recently become possible to screen clones for the presence of nonsense mutations in the HGPRT gene by introducing nonsense suppressing tRNAs from yeast into the variant cells. These tRNAs are isolated from specific nonsense-suppressing yeast strains and have been characterised by their ability to suppress nonsense codons in *in vitro* protein synthesising systems (R. Gesteland, University of Utah, Salt Lake City). They can be introduced into mammalian cells either by direct microinjection using glass capillaries (Graessman & Graessman *Proc. natn. Acad. Sci. U.S.A.* **73**, 366; 1976) or, on a much larger scale, by being trapped within red blood cells which are then fused to the recipient cells (Kaltoft *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 2793; 1976). Preliminary experiments using the first procedure have shown, by autoradiographic measurement of ^{3}H-hypoxanthine incorporation combined with videotape recording of which cells were injected, that yeast nonsense suppressors cause a temporary phenotypic reversion when introduced into certain HGPRT-deficient cell lines (Celis). The second procedure can also be used to bring about *in vitro* suppression of nonsense codons (Capecchi) but is less readily applicable to the screening of many clones for nonsense mutants. It should also be possible to introduce frameshift suppressor tRNAs, isolated from frameshift suppressing strains of yeast, into variant cells, but these tRNAs may be too specific in their action to provide a selective system for the majority of frameshift mutants.

By fusing two mammalian cell lines, each carrying the same type of mutation but in different structural genes, it will be possible to isolate a hybrid cell carrying two identical mutations. In such a cell there will be a very low rate of simultaneous spontaneous reversion to the wild-type phenotype in both genes and the stage will be set for isolating the first true suppressor mutations of mammalian cells. Informational suppressors in higher organisms are already being characterised in *Drosophila melanogaster* and the nematode *Caenorhabditis elegans*. In the latter, Waterston and Brenner (*Nature* **275**, 715; 1978) have described a suppressor of the paralysed paramyosin-deficient E1214 mutant that acts on a range of alleles and partially restores paramyosin in worms homozygous for the mutation. The paramyosin synthesised as a result of the action of this suppressor seems to be identical to the wild-type protein. In *Drosophila* the existence of informational suppression is well characterised genetically, although in no single specific instance is the biochemical mechanism established.

Some disorganised muscle mutants of the nematode *Caenorhabditis elegans* produce a shorter myosin heavy chain than normal (A. R. MacLeod *et al. J. molec. Biol.* **114**, 133; 1977). After cyanylation of the wild-type and mutant proteins with ^{14}C-labelled cyanide, a reaction which breaks the myosins at their very infrequent cysteines and labels the N-termini of the fragments with ^{14}C, and gel fractionation of the products of partial cleavage of each of these fragments with cyanogen bromide, it is possible to distinguish nonsense and deletion mutants. In a nonsense mutant only the largest labelled fragment from one of the cyanylation products will have a reduced mobility relative to the fragments from the wild-type myosin, whereas in most deletion mutants several fragments show a diminution in size corresponding to the size of the deletion (S. MacLeod, University of Cambridge). Besides providing a way of mapping deletion mutants within the coding region, this approach can also be used to detect suppression of nonsense mutations in the genes for the heavy chain of myosin. □

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A hundred years ago

NEWS from Panama states that the volcano Cotopaxi is in a state of violent activity. Its crater is surrounded by ice and snow but the clouds of ashes and smoke rising from it can be seen even at Guayaquil on the shores of the Pacific.

A VIOLET coloured meteor, with a reddish train, was seen at Stanislas, Austria, on the evening of the 24th in the Great Bear and moving in a northerly direction. It is described as thrice the size of Jupiter.

. . . it is stated that carrier pigeons are "being turned to useful account" in a new direction in Germany, for Consul Ward writes to the Foreign Office "that the successful results attained by the establishment of communication between the two Eider lightships and the Port of Tonning, in Schleswig, by these means has led to its organisation" elsewhere. This mode of communication is, however, not new, as carrier pigeons were employed early in this country as a means of communication with the Bell Rock Lighthouse, as mentioned in my late father's "Account" of that work. The pigeons passed between the lighthouse and the shore—a distance of eleven miles in eleven minutes. The employment of these birds, however, was, I suppose, found to be more curious than convenient for they have long since ceased to be employed.

From *Nature* **19**, 7 November, 5, 17; 1878.

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BOOK REVIEW SUPPLEMENT

Constraints on human behaviour

John Maynard Smith

On Human Nature. By E. O. Wilson. Pp.246. (Harvard University Press: Cambridge, Massachusetts, and London, 1978.) $12.50; £7.85

THIS, the author tells us, is "not a work of science; it is a work *about* science". Its thesis is that, in the coming period, the social sciences will be transformed by contact with evolutionary biology, in the same way as, in the immediate past, biology has been transformed by contact with chemistry. Human nature, like the nature of ants, hunting dogs or primroses, is the product of evolution by natural selection. It does not vanish with the appearance of culture, but remains as a constraint on the kinds of ways in which people can behave, and the kinds of societies which they can construct. Knowing this, he argues, it is both possible and useful to discover the nature of these constraints.

Like Wilson's earlier *Sociobiology*, the book is bound to arouse controversy; indeed, it is intended to do so. I hope that criticism will be of things Wilson does say, and not of things he does not. As most criticism will come from previously entrenched positions, it may help if I briefly describe my own. On many points I agree with Wilson. I do see recent advances in evolutionary biology, particularly in the field of behaviour, both as important in themselves, and as bound to give rise to renewed speculation about the relevance of biology to sociology. I do not think it sensible to see man as the behaviourists see him, a *tabula rasa* on which culture can write what it will. I do regard our higher faculties, such as our ability to construct mathematical arguments or to have religious experiences, as requiring an explanation in terms of natural selection.

Granting all this, however, I was not persuaded by Wilson's *Sociobiology* that biologists have all that much to contribute, for two main reasons. First, even if there are "human universals" which act as constraints on our behaviour, their nature will be discovered by psychologists and anthropologists; we biologists can only make encouraging noises from the sidelines. Second, human societies change far too rapidly for the differences between them to be accounted for by genetic differences between their members (Wilson, I think, would accept this). Hence, as differences are what we are primarily interested in, there is little an evolutionary biologist can say.

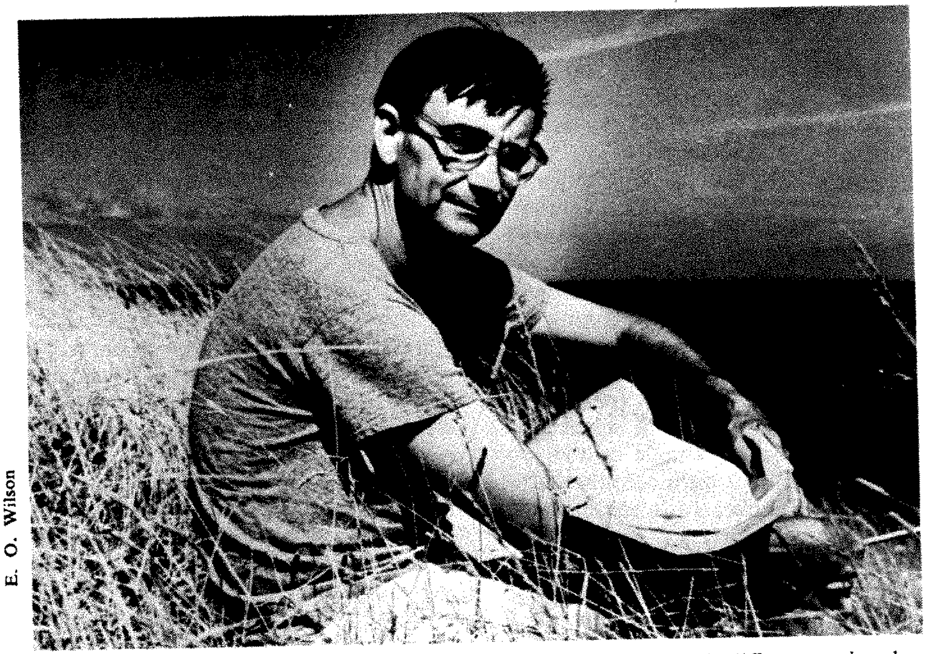
What, then, of *On Human Nature*? First, and most important, I found it entertaining and stimulating. I still have my doubts about how much progress can be made along these lines, but I would like to see it read both by students and professors of sociology. It would stimulate their imaginations to learn, on the one hand, that human societies represent only a sample of the animal societies that are possible, and on the other, that features of society they may have thought unique to man are found in other animals.

Wilson has defined his attitude to "genetic determination" more carefully in this book. Interestingly, he recommends Waddington's concept of canalisation as a paradigm for thinking about human nature and nurture. When he speaks of a trait as innate, he does not mean that it will develop in a fixed way regardless of the environment. He means only that a trait will develop in a rather constant manner despite wide changes in the environment, while accepting that a sufficiently drastic change in upbringing (or perhaps a sufficiently early one) might produce a profound change in outcome. By saying that characteristics are innate, he means only that we acquire some characteristics very readily and others only with great difficulty.

How then are we to recognise what is innate? Wilson reminds us that, classically, geneticists do not ask whether characteristics are genetically determined, but whether differences are. The difference between blue eyes and brown is genetic, and between speaking English and French is not, but it is meaningless to ask whether eyes or languages are genetic or environmental, as both require both genes and an environment. This has seemed to me a serious difficulty with the search for innate human universals. Wilson's answer is that we can look at the difference between all humans on the one hand, and all chimpanzees, say, on the other. Thus, if most human beings avoid committing incest this can be regarded as innate, because there are other animals (not, as it happens, chimpanzees) which regularly do commit incest. The fact that incest does occur in our species does not prove that there is no genetically determined tendency to avoid it, because Wilson is not using the term "genetically determined" to mean "certain", but only "likely in most environments".

This attitude can be further illustrated by his chapter on aggression. It opens "Are human beings innately aggressive? This is a favourite question of college seminars and cocktail party conversations, and one that raises emotion in political ideologues of all stripes. The answer to it is yes." Now the reason this opening raises emotion in political ideologues (including this one) is that it will be taken to mean that human beings are aggressive come what may, that war is inevitable, and that it is therefore a waste of time to work for peace. But it turns out that Wilson does not mean anything of the kind. By saying that we are innately aggressive, he means only that we have shown aggressive behaviour, including warfare, in most, but not all, of the cultural environments in which we have so far found ourselves. He emphasises that the word aggression has been used to describe many disparate patterns of behaviour. He ends the chapter with a discussion of how we might circumvent our tendency to be violent towards one another. Given that these are his views, I think that the opening words of the chapter are unfortunate. They will certainly provoke controversy, but it is likely to be of that singularly useless type which takes place between people who do not understand one another. Rather than speak of traits being innate, it would be more sensible to ask: What are the circumstances, genetic and environmental, in which a particular trait appears, and how can it be modified?

Wilson's attitude can perhaps best be illustrated by his treatment of human sex differences. All known human societies show some economic sex role differentiation, which shows considerable bias, in that some roles (for example, making tools) are performed in most societies by men, and others (for



E. O. Wilson

Photo: D. I. Crossley/AP

example, making pots) by women, although the bias is never absolute. Both the role differentiation and the bias in it call for an explanation. There seem to be two possibilities. The first is that there is a physical difference between men and women (women bear and feed babies; men are stronger and swifter) and the roles adopted are a direct consequence of this. The second is that there are innate cognitive and temperamental differences between men and women predisposing them to these roles. As technology is reducing the importance of purely physical differences, it would be easier to abolish role differentiation, should we so wish, if the first hypothesis is true.

Wilson's position, in brief, is that there probably are slight cognitive and temperamental differences between the sexes, but that sexual exploitation has been exaggerated as society has become more complex. He makes it quite clear that whether such differences exist or not need not determine our aims in present-day society. We could choose to exaggerate existing differences, or to give equal opportunity (in which case, he believes, we would not achieve statistical equality in role performance), or we could deliberately aim at statistical equality. Thus, he makes it clear (as was not clear, at least to me, in *Sociobiology*) that he does not subscribe to the view that what evolution has produced is morally right.

In general, his attitude seems to me sensible. The physical differences between the sexes are clearly a product of natural selection, and provide a clue to the breeding system of our recent ancestors. As an evolutionary biologist, I would find it odd if there were not also psychological differences; if economic role differentiation is ancient, as it may well be, then selection favouring a psychological difference is also ancient. Wilson offers two types of evidence for such innate differences. One, that girls exposed to masculinising hormone in the womb often behave in ways more typical of boys, is relevant and suggestive. The other, concerning behavioural differences between small children, is less persuasive; even if children are treated alike, they know their own sex, and will imitate adult behaviour.

This book will arouse controversy on many separate issues. Some will criticise it on the grounds that any emphasis on man's biological nature will be used to defend the *status quo*. I think such criticism mistaken. Women have been monstrously misused; it is natural that many of them are angry and frustrated. But the cause of women will not be helped by refusing to think dispassionately about the nature of sex differences.

There are many other issues. To what extent is a causal, scientific approach—whether sociobiological, Marxist, functional or whatever—appropriate to the analysis of human affairs? If the religious faculty, like the excretory, is a mere product of natural selection, where can we look for the basis of our moral beliefs? To me, the most interesting question is how far evolutionary biology can contribute to the human sciences. As I have explained, I am a doubter. But I have been wrong on this issue before. Ten years ago I regarded incest avoidance as an entirely cultural phenomenon; only a bigot could hold this view today. I hope Wilson's book will be read and debated; I hope, too, that during the debate each side will listen to what others are saying. ☐

John Maynard Smith is Professor of Biology at the University of Sussex, UK.

Rejoicing in multifarious nature

Richard Dawkins

Ever Since Darwin: Reflections in Natural History. By Stephen Jay Gould. Pp.285. (Burnett Books/André Deutsch: London; Norton: New York, 1978.) £5.50.

"The author shows us what is revealed when we remove the blinkers which Darwin stripped from biology a century ago." Some overkill there, or an excitingly paradoxical striptease technique? The first essay in the book discusses Darwin's own coyness in not revealing his theory until 20 years after he thought of it, and I shall return to this. The quotation from the jacket blurb gives a false impression, for Stephen Gould's writing is elegant, erudite, witty, coherent and forceful. He is also, in my opinion, largely right. If there are elements of paradox and overkill in Dr Gould's intellectual position they are not to be found within these covers. *Ever Since Darwin* is a collection of essays which first appeared as a regular monthly column in a magazine called *Natural History*. Skilfully edited to flow in eight main sections, the 33 essays, of which I can mention only a sample, reinforce my feeling that scientific journalism is too important to be left to journalists, and encourage my hope that true scientists may be better at it than journalists anyway.

Gould's collection begins to bear comparison with P. B. Medawar's immortal *The Art of the Soluble* (Methuen, reprinting). And if his style does not quite make the reader chortle with delight and rush out to show somebody —anybody—the way Medawar's does, Gould is to be thanked for some memorable lines. No doubt puritan killjoys of Science for 'The People' will denounce the vivid and helpful anthropomorphism in "Reproduce like hell while you have the ephemeral resource, for it will not last long and some of your progeny must survive to find the next one" (p94). But on second thoughts they may be too busy plotting the abolition of slavery in ants (p265), or brooding over the deviationism of "Natural selection dictates that organisms act in their own self-interest . . . They 'struggle' continuously to increase the representation of their genes at the expense of their fellows.

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And that, for all its baldness, is all there is to it; we have discovered no higher principle in nature" (p261). My only recurrent complaint against the style concerns the journalistic adjectival phrase before names: "English biologist J. B. S. Haldane probably anticipated every good idea that evolutionary theorists will invent this century".

Ever since Darwin we have known why we exist and we have known at least how to set about explaining human nature. I agree that natural selection is "the most revolutionary notion in the history of biology" (p23) and I would toy with substituting 'science' for 'biology'. Childishly simple as it is, nobody thought of it until centuries after far more complicated ideas had become common currency, and it is still the subject of misunderstanding and even apathy among educated people. A microcosm of this historical enigma is the subject of Gould's first essay. Just as humankind waited centuries longer than our hindsight deems necessary before discovering natural selection, so Darwin delayed his own publication 20 years after he first thought of the theory in 1838. Gould's explanation is that Darwin was afraid of the psychological implications of his idea. He saw, what Wallace would never admit, that the human mind itself must be a material product of natural selection. Darwin, in fact, was a scientific materialist—a biological determinist. And we know how biological determinists can expect to be treated by their colleagues. No wonder he delayed publication.

In another essay Gould is encouraged by the genetic closeness of humans and chimpanzees to speculate that "inter-breeding may well be possible". I doubt it, but it is a pleasing thought and Gould surely exaggerates when he rates it "the most . . . ethically unacceptable scientific experiment I can imagine" (p54). For my ethics, far less acceptable experiments are conceivable, and actually done in animal physiology laboratories every day, and a chimp/human hybridisation would provide exactly the come-uppance that 'human dignity' needs. Gould is, in general, rather good at puncturing human speciesist vanity, and in particular he will have nothing to do with the myth that evolution represents progress toward man. This scepticism informs his valuable account of "Bushes and ladders in human evolution", and fires his scorn for attempts to rank human races as primitive or advanced. He returns to the attack on progress in the very different guise of the theory of orthogenesis, the idea that evolutionary trends have their own internal momentum which eventually drives lineages extinct. His telling of the classic Irish Elk story gains freshness from his intimacy with the fossils of the Dublin Museum and gives the lie to the myth that palaeontology is dry and dull. His conclusion that the proverbially top-heavy antlers were important in social life is surely right, but he may underestimate the role of within-species competition in driving species extinct. Large antlers could directly have caused the extinction of the Irish Elk while, at the same time, right up to the moment of extinction, individuals with relatively large antlers were out-reproducing individuals with relatively small antlers. I would like to see Gould come to terms with the "orthoselective" impact of "arms races" both between and within species. He seems to approach this in his essays on the "Cambrian explosion".

Ooh-ah biology can be sold for its intrinsic fascination, but it is much better used to make a point. Gould tells us about a fly that eats its mother from the inside, about seventeen-year cicadas and 120-year bamboos, and about uncanny fish-decoying mussels. He employs the useful trick of first opening the reader's mind by boggling it, then filling it with an important biological principle. One principle I would like to have heard more on is that of the limitation of evolutionary perfection: "Orchids are Rube Goldberg machines; a perfect engineer would certainly have come up with something better" (p91; Rube Goldberg is the American Heath Robinson). My own favourite example, inherited from an undergraduate tutor, is the recurrent laryngeal nerve. It starts in the head, goes down into the chest, loops round the aorta, then goes straight back into the head again. In a giraffe this detour must be wasteful indeed. The human engineer who first designed the jet engine simply threw the old propeller engine out and started afresh. Imagine the contraption he would have produced if he had been constrained to 'evolve' his jet engine by changing a propeller engine one bit at a time, nut by nut and bolt by bolt! While on the problem of perfection, I think Gould exaggerates the relevance of 'neutral mutations'. Molecular geneticists are understandably interested in DNA changes as molecular events, and any that have no effect on protein function may reasonably be called neutral mutations. But to a student of whole organism biology these are less than neutral: they are not, in any interesting sense, mutations at all! If the molecular neutralists are right, their kind of neutral mutation will for ever be hidden from the field biologist and from natural selection. And if a field biologist actually sees variation in phenotypes, the question of whether

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that variation could be selectively neutral cannot be settled in the biochemistry laboratory.

Several essays touch on aspects of the relationship of Darwinism to human society and politics. There is much humane good sense here and I agree with most of it. Although 'sociobiology' is inspiring excellent research, Gould is right that it has also lead to some second-rate bandwagoneering. "But was there ever dog that praised his fleas?" asked Yeats. Perhaps a dog may be held responsible for the fleas that he sheds, but only to a small extent. At the AAAS meeting in Washington this year, Gould and I witnessed an organised attack on his most distinguished Harvard colleague. Gould well deserved his ovation for the apt Lenin quote with which he disowned the rabble. But as he watched those pathetic fleas ineffectually hopping around the stage chanting, of all things, "genocide", did he wonder with a little itch of conscience on which dog they had been sucking?

The Epilogue is forward-looking, and whets our appetite for the Volume 2 which I earnestly hope will be forthcoming. One theme which I know Gould has already carried further in his *Natural History* column is his dislike for "the ultimate atomism" of regarding organisms as "temporary receptacles . . . no more than instruments that genes use to make more genes like themselves" (p269). In describing this as "metaphorical nonsense", Gould underestimates the sophistication of the idea, first cogently expressed in its modern form by George C. Williams (*Adaptation and Natural Selection*, Princeton University Press, 1966 pages 22–25 and 56–57). The dispute is largely semantic. Inclusive fitness is defined in such a way that to say "the individual works so as to maximise its inclusive fitness" is equivalent to saying "the genes work so as to maximise their survival". The two forms are each valuable for different purposes. Both contain an element of personification; it is dangerously easier to personify organisms than to personify genes. The gene selection idea is not naïvely atomistic, as it recognises that genes are selected for their capacity to interact productively with the other genes with which they are most likely to share 'receptacles'; this means the other genes of the gene pool, and the gene pool may therefore come to resemble a "homeostatically buffered system" tending to return to (one of) its evolutionarily stable state(s). Irrevocable determination by genes is no part of the idea, nor is anything remotely approaching a "one gene one trait" mapping from genotype to phenotype. In any case it has nothing to do with "supreme confidence in universal adapttion" (p269), which is as likely to be found among devotees of "individual selection" or "species selection".

"I will rejoice in the multifariousness of nature and leave the chimera of certainty to politicians and preachers" (p271): a resounding conclusion to a stimulating book—the work of a free and imaginative scientific mind. The final, sad paradox is this. How can a mind capable of such rejoicing, open enough to contemplate the shifting splendour of three thousand million years, moved by the ancient poetry written in the rocks, how can such a mind not be bored by the drivelling ephemera of juvenile pamphleteers and the cold preaching of spiteful old hardliners? No doubt they are right that science is not politically neutral. But if, to them, that is the most important thing about science, just think what they are missing! Stephen Gould is well qualified, and strategically placed, to strip away even those dark blinkers and dazzle those poor unpractised eyes.

Richard Dawkins is Lecturer in Zoology at the University of Oxford, UK.

Evolutionary advantages of sex

Alan Robertson

Evolution of Sex. By John Maynard Smith, Pp.222. (Cambridge University Press: Cambridge and London, 1978.) Hardback £9.50; paperback £2.95.

"WHAT song the syrens sang or what name Achilles assumed when he hid himself among women, though puzzling questions, are not beyond all conjecture", and so it is with the evolution of sex. Maynard Smith takes up again points which were first discussed by Darwin and, often with tantalising brevity, in Fisher's classical book of 1930.

Maynard Smith himself added a most important point to the argument in 1971 that in sexual species there is an immediate selective advantage for a gene which would allow females to reproduce parthenogenetically. In a sexual population of fixed size, each individual has on the average two progeny in the next generation and each gene contributes on average one copy. Suppose a mutant occurs which, without reducing the numbers or the vigour of the progeny of a female allows her to reproduce parthenogenetically. She would still contribute two progeny but all their genes would come from her so that the new mutant would be represented on average by two copies in the next generation. The mutation would then spread rapidly through the population.

Thus, parthenogenesis passes the first test to be applied to any potential evolutionary change in a species—will the genes which favour it have in consequence a selective advantage. Such mutants are clearly possible. *Daphnia*, for instance, has adopted a strategy of cyclical parthenogenesis, breeding parthenogenetically in summer and sexually as winter approaches. A strain of turkeys is known in which a proportion of unfertilised eggs hatch—not very usefully in this case because all such progeny are male.

But there are further tests. Will species which have adopted this strategy be able to compete against their parental sexual species if the environment suddenly changes (for which the genetic variation in the species at any time is important) or when the environment is slowly changing over thousands or millions of generations, so that the species depends for successful change on its ability to accumulate new useful mutations, and also to get rid of the harmful ones. These are properties of the species as a whole rather than of its component individuals, so that competition is between populations—'group selection'.

A new and successful parthenogenetic species, having already passed the first test in its present environment will be less likely to pass the second two tests, as Muller showed. It will clearly be more genetically uniform at any time. The majority of new mutants will be harmful and the species will be less able to tolerate them than will a sexual species. This is Muller's famous "ratchet mechanism". He showed that asexual populations below a certain critical size, depend on the overall mutation rate to harmful genes and their average selective disadvantage, will decline in fitness, essentially because of continued chance of loss of the fittest genotype.

Muller also showed that a sexual population is very much more efficient in accumulating new advantageous mutants, at least in situations in which many such selective substitutions are occurring at the same time. In an asexual species, the only way that two advantageous mutants can be combined in the same clone is by mutation in that clone, whereas in a sexual species recombination is possible of mutations which originally occurred in different individuals. Muller presents these two arguments separately but I suspect that, as Felsenstein has pointed out, they really are aspects of the same process. Thus, we have two ways in which group selection will in the long term favour sexual compared with parthenogenetic species.

Maynard Smith's approach is to present first the theoretical aspects of questions and second to examine the available evidence in both plants and animals. He concludes that, though group extinction must play an important part in maintaining sexual reproduction, there must also be some short term advantages for sex.

It may not have escaped the reader that all the long term advantages of sex come from the independent assortment of chromosomes and that they therefore apply equally well to the maintenance of recombination. There is good evidence that the amount of crossing over on a given region can be altered by selection so that the raw material for natural selection is there. Theory would suggest that in a large population in a uniform environment, natural selection would in the short term favour a reduction in crossing-over. This requires epistatic interactions affecting fitness between genes at loci fairly close together on the chromosome. The arguments about the long term effects are the same as I have indicated for sex itself—indeed as initially presented the arguments referred to recombination rather than to sex.

Are there any short term advantages for recombination? Two are presented. The first, due to Maynard Smith himself, is in terms of competition between sibs. He shows that, if the environment varies widely from year to year and is described by several different variables, an individual of a given genotype in whose progeny there is segregation has a higher chance of producing successful offspring than one which does not. This he confirms in a model with five environmental features and five loci, one locus controlling adaptation to one feature of the environment. He finds clear selective advantage for higher recombination. The model, however, turns out to be not very robust to changes in the assumptions.

There is an alternative explanation, presented in a way that I found very obscure, even though I am an author of what is said to be the key paper. After much struggle, I arrived at an understanding as follows. Muller showed that, in the long term, a sexual species is better at accumulating advantageous new mutations and getting rid of bad ones. With no recombination, two useful mutations which have occurred in different individuals cannot be combined. Looking at the whole population, there is then a complete absence of gametes containing both. Felsenstein showed that, in one special case, Muller's argument could be extended to short term selection—gametes which had arisen by recombination would be fitter than those which had not.

Our paper dealt with selection in finite populations, and we showed that, if two advantageous linked mutants are under selection at the same time, they tend to block each other's progress, because, under the joint action of selection and random sampling, there is in such populations a shortage of gametes containing both mutations. Increased recombination then speeds up selection. But Muller says nothing about population size. On reflection, a critical part of his argument concerns the occurrence of new mutants as units—the total number of copies of any mutant in the population is certainly finite at critical stages. So perhaps our statement contains Muller's as a special case. Felsenstein was able to confirm by simulation that recombination would be selected for in finite populations in which new mutants, either advantageous or harmful, are occurring. I should say that I remain a little uneasy that such a major problem, the evolutionary implications of recombination, turns out to depend on such a 'second-order' mechanism.

Using the same general approach, the final third of the book deals with a series of secondary but related problems, such as the evolution of hermaphroditism, the avoidance of inbreeding, the evolution of anisogamy and the sex-ratio, and finally, thrown in for good measure, a discussion of optimal mutation rates.

I found the book stimulating in that it took me down many paths that I am not used to treading. It contains a fair amount of mathematics, which is almost all of the 'nuts and bolts' kind. But I did find it difficult. There could of course be three reasons for this—the author, the nature of the material, and finally the reviewer. I must say that I was a little taken aback when, having completely failed to penetrate a paragraph in one of the papers referred to, the authors then proceeded; "but the argument cannot really be this simple." ☐

Alan Robertson is Professor in the Department of Genetics at the University of Edinburgh, UK.

Shaking off the magic

P. B. Medawar

Magic, Science and Civilization. By Jacob Bronowski Pp.104. (Columbia University Press: New York, 1978.) $8.70.

BRONOWSKI believes that a radical and irreversible complex of changes in the character of civilised life took place between 1500 and 1700: that which followed the eruption *(sic)* of science into western civilisation. It is characteristic of the rather lazy and flabby character of this book that although he clearly does not mean it he refers to the complex of changes as a 'step' and likens it to a mutation. The "prime contemporary issue" is "the place of science in the total field of knowledge and that of human values as we have to reformulate them in this century".

Bronowski believes that round about 1600 (give or take a century) it became possible for the first time to propound a unitary theory of the world: "to see the world as a whole and from a single core or type of explanation". He mentions grave and well recognised objections to this view, without in my opinion, countering them.

Bronowski is rather good on magic, of which his definition is admirably simple: "It is the view that there is a logic of everyday life, but there is also a logic of another world. Science is distinguished from magical views by the fact that it refuses to acknowledge a division between two kinds of logic . . . There is only one logic; it works the same way in all forms of conduct and it is not carried out by any kind of formula . . .".

Alchemical notions had a profound influence on the development of chemistry and chemical technology and in comparatively recent years it has become clear from the research of Frances Yates, Paolo Rossi and Charles Webster that magical, supernatural and literally scriptural notions played a most important part in the thought of many of those whom we think of as pioneers and champions of modern science; in particular one thinks of Frances Yates's discernment of Rosicrucian and hermetic influences in, for example, the writing of Francis Bacon and his contemporaries here and abroad. Dr Joseph Glanvill, FRS, for example, was an ardent believer in witchcraft and at the same time a champion of the Royal Society and all it stood for. This division of mind must have been most unsettling: it is no wonder that a *Tempest* raged in Prospero's heart.

Modern science has shaken off magic but Bronowski is very clearly

An Historical Geography of England and Wales

edited by R. A. Dodgshon and R. A. Butlin

October 1978, paperback edition: xx + 450pp., £6.80/$14.00 0.12.219252.4
hardback edition: xx + 450pp., £14.80/$30.65 0.12.219250.8

During the last decade, historical geography has undergone important changes in both method and content, which greatly enhance its contribution to geographical studies. As it is now an integral part of the geography syllabus in many universities and colleges, there is an urgent need for a basic foundation text for undergraduates which incorporates these changes. This multi-authored, single-volume textbook will cater for just such a need. It reflects the important developments which have taken place in the discipline, not only in terms of ideas and understanding but in its original style of approach — it is perhaps the first to concentrate on an interpretative and explanatory angle as regards spatial patterning.

Seeing and Hearing and Space and Time

N. O'Connor and B. Hermelin

November/December 1978, viii + 158pp., £6.80/$14.00 0.12.524150.X

This book discusses the interrelationship between perceptual and cognitive processes, describing recent experimental work carried out by the authors with sensorily and cognitively impaired children. It reports and compares the coding strategies of blind, deaf, autistic, subnormal and normal children presented with different tasks, with the underlying hypothesis that sensory deficits will affect cognition, and cognitive impairment will in turn influence perceptual organization. The study groups were chosen to contain children of different intellectual attainment as well as different diagnoses, so that the question-begging explanation of cognitive deficit in terms of intelligence might be avoided and the components of the deficit properly analysed.

Reversibility of Female Sterilization

edited by Ivo Brosens and Robert Winston

November/ December 1978, x + 194pp., £7.50/$15.50 0.12.136080.6

Sterilization has become the most extensively used method of contraception, and at the same time the demand for reversal of sterilization has also increased. In view of this increasing demand, a number of leading specialists in the field were invited to discuss different aspects of potential reversibility of female sterilization at the first international symposium on the subject, held in Leuven, Belgium, in June 1977. This book comprises both the papers presented and the discussions held at the symposium. Although dealing mainly with technical aspects of reversal, the papers also include discussions on the scientific background to fallopian tube function (physiology, pathology and ultrastructure), different methods of sterilization with particular reference to their reversibility, and psychological and sociological aspects of reversal and the demand for it. *Published in New York by Grune & Stratton (ISBN 0.8089.1150.3)*

Rice in Africa

edited by I. W. Buddenhagen and G. Persley

December 1978/January 1979, xvi + 356pp., £12.50/$25.75 0.12.139350.X

Rice is traditionally thought of as an Asian crop and indeed a large proportion of the world's rice is grown there. However, certain African countries also have a long history of rice production and its cultivation is increasingly being introduced into others. It is important therefore that research and development specific to Africa is co-ordinated and encouraged if production is to be improved and best use is made of resources. To help stimulate communication in this area a conference was organised by the Institute of Tropical Agriculture in collaboration with the International Rice Research Institute, the Institut de Recherches Agronomiques Tropicales, and the West African Rice Development Association. This volume is the result of the conference.

New Trends in Bio-Inorganic Chemistry

edited by R. J. P. Williams and J. R. R. F. da Silva

December 1978/January 1979, x + 490pp., £12.50/$25.75 0.12.755050.X

This book concentrates on aspects of inorganic chemistry and biology which have often been overlooked. In the past, the main stress in bio-inorganic chemistry has been on metals such as iron, copper and magnesium. Here, by contrast, the overall objective is to indicate to the reader that a much larger number of inorganic elements are involved in bio-inorganic chemistry. Consequently, one major emphasis is on the biological roles of elements such as silicon and phosphorus. Undergraduates and graduates in biochemistry and inorganic chemistry will find this a stimulating approach to the subject and a valuable reference work. The book will also be useful to students of medicine who have an interest in trace elements and mineral metabolism.

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P.O. Box 300, North Ryde, NSW 2113, Australia

Relativistic Quantum Fields

C. Nash

November/December 1978, x + 224pp., £15.00/$31.00 0.12.514350.8

In the last ten years there have been a number of attempts to make progress in various problems in quantum field theory. This has resulted in the creation of several new techniques which are now important for a research worker doing calculations in field theory. Among these techniques are the short distance expansion of Wilson, the dimensional regularization method, and the renormalization group methods. This book is an attempt to present these techniques in a form comprehensible to postgraduate students in their first and second years of research.

Methods in Microbiology

Volume 11

edited by T. Bergan and J. R. Norris

December 1978/January 1979, xvi + 314pp, £16.00/$33.15 0.12.521511.8

Interest in the factors controlling the spread of pathogenic bacteria in both human and animal populations has led, in recent years, to the development of various techniques for the characterization of isolates from epidemics. These take many forms. Biochemical tests, serological analysis, phage and bacteriocin typing are particularly important. Volumes 10-13 of ***Methods in Microbiology*** collect together, for the first time, the methods used in identifying all major human and animal pathogenic bacteria of epidemiological importance. The attention of practical detail will enable the methods to be followed in the laboratory, and it is hoped that this will lead to increased uniformity of method around the world.

Advances in Physical Organic Chemistry

Volume 16

edited by V. Gold and D. Bethell

December 1978/January 1979, viii + 274pp., £16.80/$34.75 0.12.033516.6

This is a well-established series for the publication of authoritative reviews on the methods and results of the application of quantitative physical and mathematical methods to organic chemistry.

Contents

John H. Ridd: Diffusion control and pre-association in nitrosation, nitration and halogenation. **John M. Tedder and John C. Walton:** Directive effects in gas-phase radical addition reactions. **John Albery and Maurice M. Kreevoy:** Methyl transfer reactions. **John O. Williams:** Electrical conduction in organic solids. **Stefan Berger:** Nuclear magnetic relaxation: recent problems and progress. Author index. Cumulative index of authors. Cumulative index of titles.

Rational Quadratic Forms

J. W. S. Cassels

November/December 1978, xvi + 414pp., £17.50/$36.25 0.12.163260.1

This monograph presents the main themes of the theory of quadratic forms at a level that can be appreciated by a wide variety of readers. The first half of the book develops the classical theory of rational and integral equivalence for quadratic forms with rational or rational integral coefficients. Full use is made of the insight and clarity provided by the use of p-adic numbers and of Witt's theory of quadratic spaces. The second half contains treatments of spinor genera, of reduction theory and automorphs, and of the composition of binary forms.

Proceedings of the Serono Symposia No. 14

Recent Progress in Andrology

edited by A. Fabbrini and E. Steinberger

December 1978/January 1979, xiv + 506pp., £17.60/$36.50 0.12.24.247350.7

Andrology is a relatively new discipline which has developed from inter-relationships between disciplines once regarded as isolated from each other. The endocrinologist, urologist, psychologist, neurologist, seminologist, paediatrician, geneticist, and many other specialists realised that their specialised knowledge converged in the field of clinical and experimental physiology and pathophysiology of the male. One of the first symposia to be organised around this multidisciplinary concept was held in L'Aquila in April 1977, the proceedings of which are presented in this volume. It covers all aspects of andrology including neuroendocrinology, testicular pathology and physiology, and antifertility agents and therapy.

Chemical Oceanography

2nd Edition Volume 7

edited by J. P. Riley and R. Chester

November 1978/January 1979, xvi + 508pp., £19.60/$40.50. 0.12.588607.1

Reviews of the First Edition "*. . . a most comprehensive review of the subject, and a work of real significance to the advanced student and research worker, and of interest to chemists, biologists and geologists in associated fields.*"
Chemistry in Britain.

Contents

E. J. W. Jones: Sea-floor spreading and the evolution of the ocean basins. **Ted C. Moore, Jr. and G. Ross Heath:** Sea-floor sampling techniques. **William M. Sackett:** Suspended matter in sea-water. **Walter W. Berg, Jr. and John W. Winchester:** Aerosol chemistry of the marine atmosphere. **Bernd R. T. Simoneit:** The organic chemistry of marine sediments. **Karl K. Turekian and J. Kirk Cochran:** Determination of marine chronologies using natural radionuclides. **S. R. Aston:** Estuarine chemistry. **L. D. Mee:** Coastal lagoons.

Advances in Parasitology

Volume 16

edited by W. H. R. Lumsden (Senior editor) and J. R. Baker

December 1978/January 1979, xii + 376 pp., £18.80/$38.75 0.12.031716.8

This latest volume, the first compiled by the new editors, continues the series along the same lines as under the editorship of the late Professor Dawes. In the past, the series has been widely recognised as a leading publication in the field, and has gained a high reputation for its in-depth and original reviews on a variety of subjects within the ambit of parasitology. The scope of future volumes will remain much the same as in the past, with the addition of some more medically orientated subjects, reflecting the bias of the new editors.

Proceedings of the Serono Symposia No. 18

The Endocrine Function of the Human Adrenal Cortex

edited by V. H. T. James, M. Serio, G. Guisti and L. Martini

December 1978/January 1979, x + 628pp., £19.50/$40.35 0.12.380160.5

This book is a record of the proceedings of a symposium held in Florence in October 1977. The meeting brought together a selection of speakers, many of whom have made important and fundamental contributions to the understanding of adrenal cortical functionings in recent years. This volume presents both a comprehensive review and details of current research on the many aspects of the functioning of the adrenal cortex for all those interested in human endocrinology.

Bioluminescence in Action

edited by P. J. Herring

November/December 1978, xxvi + 570 pp., £25.00/$51.75 0.12.342750.9.

The study of bioluminescence, the emission of light by living organisms, is not only rewarding in its own right but it has also provided invaluable tools and concepts in other areas of research as diverse as neurophysiology, space research and biological kinetics. *Bioluminescence in Action* provides a review of all aspects of bioluminescence, a phenomenon whose visible expression covers the gamut from bacteria to beetles and fungi to fishes. The individual chapters are written by specialists in particular aspects of the subject, each of whom has contributed substantially to its development in recent years.

Mining Geostatistics

A. G. Journel and C. J. Huijbregts

December 1978/January 1979, x + 600pp., £32.00/$66.25 0.12.391050.1

"Geostatistics is the application of the formalism of random functions to the reconnaissance and estimation of natural phenomena." With this definition, G. Matheron introduced the term *geostatistics* in 1962 to describe a new approach to certain problems in geology and mining. This approach encompasses the notion of the contradictory 'structure' and 'randomness' characteristic of, for instance, distribution of ore grades within a deposit: it provides a coherent and efficient set of probabilistic techniques, which are available to the geologist, mining engineer or metallurgist, and which require for their understanding only the most modest acquaintance with mathematical formalism. This book is the first manual and reference work on the subject.

Photographic Techniques in Scientific Research

Volume 3

edited by A. A. Newman

December 1978/January 1979, xii + 392 pp., £35.00/$72.50 0.12.517963.4

The third volume in an acclaimed series, this book continues the aim of the series in describing photographic techniques useful to various sectors of the scientific world. Increasingly, photography is being used by scientists either as an accurate means of recording and measuring data or as a sensitive instrument for clear observation. Thus it is essential for scientists to have easy access to the advances in method and equipment which are constantly being made. Six highly-qualified specialists have provided papers on different applications of photography to scientific research. Yet readers need not confine themselves to the chapter on their specific field of interest since, by adapting the techniques described in other sections to their own particular discipline, they can discover much that will be of use to them.

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Royal Society of Medicine International Congress and Symposium series Number 2

Cardiovascular Medicine Controversies

edited by Clive Wood

November/December 1978, x + 84pp., £5.00/$10.25 0.12.763340.5 (Academic Press) 0.8089.1134.1 (Grune & Stratton)

The proceedings of an International Symposium held by the ICI Pharmaceuticals Division at the Babbage Lecture theatre, Cambridge, on 26-27 September 1977

Contents

Session I: Hypertension — Initiating mechanism. Convener's introduction. Animal models of hypertension. The role of the nervous system in hypertension. Is the kidney important in hypertension? **Session IIA:** Myocardial infarction. Chairman's introduction. Is there a biochemical basis for the management of myocardial infaraction? **Session IIB:** Sudden death. Chairman's introduction. Sudden death — thrombosis or not? **Session III:** Atherosclerosis. Chairman's introduction. The monoclonal hypothesis. Platelets and platelet mediators in atherosclerosis. Critical re-evaluation of the role of lipids.

Published jointly by The Royal Society of Medicine, Academic Press, London and Grune & Stratton (New York)

Royal Society of Medicine International Congress and Symposium series Number 4

Round Table Discussion on Gentamicin and Tobramycin

edited by R. Richardson

November/December 1978, viii + 64pp., £5.00/$10.25 0.12.587780.3 (Academic Press) 0.8089.1149.X (Grune & Stratton)

This discussion, sponsored by Schering Corporation, held in London, 13 February 1978, is based on presentations made at the Tenth International Congress of Chemotherapy in Zurich. A worldwide group of experts in aminoglycoside therapy who were present at Zurich discuss their data and their interpretation of these findings.

Contents

Introduction. **Part I:** Microbiology. Comparative in vitro activity of gentamicin and tobramycin. **Part II:** Toxicology. Ototoxicity. Nephrotoxicity of gentamicin and tobramycin in animal models. **Part III:** Clinical. Personal experience of gentamicin ototoxicity. The frequency of ototoxicity — a review of the literature. Personal experience with the aminoglycosides. Summary.

Published jointly by The Royal Society of Medicine, Academic Press (London) and Grune & Stratton (New York).

Special Publications of the Society for General Microbiology

Coryneform Bacteria

edited by I. J. Bousfield and A. G. Callely

October/November 1978, x + 316pp., £9.20/$19.00 0.12.119650.X

The classification and identification of the coryneform bacteria, a widely distributed and important group of organisms, have long been difficult and confused. In this book, the first in a new series of publications arranged by the Society for General Microbiology, the authors aim to show how the application of modern taxonomic methods is rapidly changing standard views on the systematics of these bacteria, and how a reliable taxonomy seems to be emerging at last.

Huckel Theory for Organic Chemists

C. A. Coulson, B. O'Leary and R. B. Mallion

November/December 1978, xvi + 182pp., £9.50/$19.65 0.12.193250.8

The late Professor Coulson virtually created Hückel Theory in its present form; future generations are therefore extremely fortunate that Drs. O'Leary and Mallion, who compiled this book, not only possessed his notes but also tape-recordings of his lectures to third-year undergraduate chemists. A gifted teacher who had taught most of the present leaders in the field of theoretical chemistry throughout the world, Professor Coulson had considered putting his lectures into book form. After his death in 1974, two of his former students and friends — Dr. Brian O'Leary and Dr. Roger Mallion — decided to carry out this task on his behalf. The result is a fascinating work which describes a field of chemistry which will always be linked with the name of C. A. Coulson.

FEMS Symposium No.5

Antibiotics and Other Secondary Metabolites

Biosynthesis and Production

edited by R. Hütter, T. Leisinger, J. Nüesch and W. Wehrli

November/December 1978, xii + 256pp., £14.40/$29.75 0.12.363250.1

Contents

The search for new secondary metabolites. Manipulation of gene expression in the development of antibiotic production. Process design and control in antibiotic fermentations. Microbial transformations — type reactions. Microbial transformations — some applications in natural product chemistry. Formation of tetracycline antibiotics. Biosynthesis of macrolide antibiotics. Rifamycin biogenesis. Developments in the chemistry and biochemistry of β-lactam antibiotics. Genetics in the development of the penicillin process. Genetics and biochemistry of cephalosporin biosynthesis. Ergot alkaloids — recent advances in chemistry and biochemistry. Genetic aspects of the formation of ergot alkaloids. Formation of ergotoxine alkaloids by fermentation and attempts to control their biosynthesis. Subject index.

Physiological Aspects of Anaesthetics and Inert Gases

A. G. Macdonald and K. T. Wann

November/December 1978, xii + 306pp., £17.50/$36.25 0.12.464150.4

Anaesthetics and inert gases affect almost every biological function, causing important changes in the physiological state of the body. This book discusses these various effects, and, in addition, provides an introduction to the scientific basis of anaesthetic action, as well as an account of the ways in which anaesthetics are used as probes in physiolcgical research. The subject has been approached by considering a wide range of physiological phenomena and discussing the effects of anaesthetics and inert gases on each of these, bringing such effects into scientific perspective.

Society for Applied Bacteriology Symposium Series No. 7

Streptococci

edited by F. A. Skinner and L. B. Quesnel

November/December 1978, xiv +416pp., £18.00/$37.25 0.12.648035.4

The genus *Streptococcus* comprises organisms which have a wide ranging impact on man and animals. This volume, which contains the papers given at the 1977 Society for Applied Bacteriology Symposium, provides a broad synopsis of the various facets of this important genus. It gives a description of the organisms themselves, their biochemical potential and the position they hold in the classification of bacteria. Many of the members are causative agents of disease, and their effects are considered in detail from the viewpoints of human medicine, dentistry and veterinary science. The streptococci also include organisms of enormous economic importance to man, such as those used in the dairy industry for the production of cheese and fermented milk products. Other species are of use in evaluating water and food pollution and some have a significant role in the digestive processes of ruminant animals. All these and other aspects of the streptococci are reviewed in this volume.

Strategies of Information Processing

edited by Geoffrey Underwood

October/November 1978, x + 456pp., £20.00/$41.40 0.12.708950.0

This is one of the most important books on experimental psychology to appear since Neisser's *Cognitive Psychology* in 1967. It not only deals with recent empirical developments in the area of cognition, but also sets out a theoretical framework in which these developments can be seen to be working. In essence, this is the distinction between cognitive structure and the strategies used to manipulate this structure around our environmental problems that are set. The notion of strategies of information processing is a powerful concept, and is used in the book to indicate how information processing theory can account for the variety and richness of an individual's behaviour whilst carrying out different tasks.

Biochemical Aspects of Plant and Animal Coevolution

edited by J. B. Harborne

October/November 1978, xviii + 436pp., £24.60/$50.90 0.12.324672.3

Our understanding of the significance of secondary plant constituents in plant and animal interactions has developed rapidly in recent years. So much so that it was considered timely to review the major developments since 1971, when the first meeting on this topic was held by the Phytochemical Society and published under the title "Phytochemical Ecology". This present volume is the result of the second meeting held in the University of Reading in 1977. Plant-insect interactions were among the first to be examined and still receive most attention. Among such studies discussed in this volume are the interactions between host plants and various insects — aphids, armyworms, bees, butterflies, grasshoppers, locusts and whiteflies.

Metal Alkoxides

D. C. Bradley, R. C. Mehrotra and D. P. Gaur

November/December 1978, viii + 412pp., £28.50/$58.00 0.12.124250.1

The metal alkoxides are a family of compounds exhibiting a wide range of physical and chemical properties. Much of the knowledge about these compounds has been obtained in the last 2-3 decades, and with the use of modern spectroscopic techniques and X-ray structural analysis, further advances are occurring rapidly. Although there have been several review articles on specific aspects of metal alkoxides, this book is the first comprehensive treatise on the subject and provides a perspective of the place of metal alkoxides in chemistry.

Biochemical Mechanisms of Liver Injury

edited by T. F. Slater

November/December 1978, approx. 850pp., £40.00/$82.75 0.12.649150.X

The book is primarily concerned with describing the variety of biochemical disturbances underlying a number of important liver injuries and diseases. Certain aspects in particular are emphasised, such as the role of metabolic activation of many hepatotoxins and hepatocarcinogens in the development of liver injury; the transient nature of such activated intermediates which makes rapid reaction studies necessary for their investigation; the importance of free radical reactions in the biochemical disturbances produced by these transient intermediates; and the multiplicity of protective mechanisms that may be applied. The book also includes some chapters dealing with the applications of relatively new techniques such as electron spin resonance, nuclear magnetic resonance.

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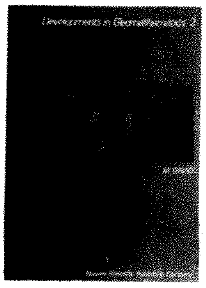

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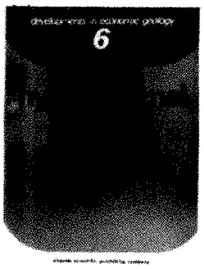

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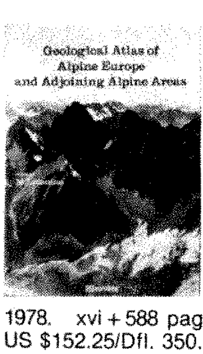

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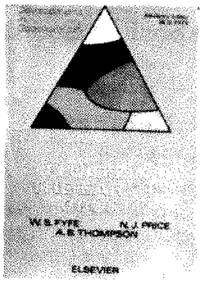

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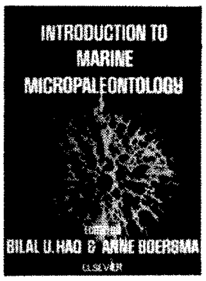

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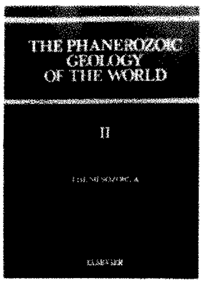

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aware of the degree to which a predilection for the magical and mysterious persists amongst laymen in the cult of various forms of contemporary irrationalism.

I thought Bronowski's book not nearly as original, profound or illuminating as one might have hoped from a man of his learning and intelligence. It gives me the impression, too, of having been rather lazily written, as if it had all come too easily to him. ☐

Sir Peter Medawar is Head of the Transplantation Biology Section of the MRC Clinical Research Centre, Harrow, UK.

Power of the scientific metaphor

M. J. Morgan

Programs of the Brain. By J. Z. Young. Pp.325 (Oxford University Press: Oxford, 1978.) £5.95.

THE conceptual problems of biological development are perhaps clarified, but made no easier of solution, by the notion that the genes encode, not so much characters in the old-fashioned sense, but rather programs or sets of instructions for development. Until this metaphor became available, the only alternative to a crude preformationist kind of innatism was a mysterious force such as Driesch's "entelechy", which would allow the developing organism to survive the vicissitudes of the environment and the embryologist's knife. The idea of the program for development is novel because it allows a complete determinism to coexist with flexibility in development. To give just one example, a vertebrate limb bud on the left side of the body can grow into a mirror image of its normal form if transplanted on to the right side of the body, because its developmental program does not specify which limb it should be, but rather how it should grow in relation to surrounding tissue. An apparently marvellous example of flexibility turns out to depend on rather an economical program.

The analogies between embryology and the empiricist–nativist controversy in psychology are very striking, as J. Z. Young makes clear in this admirable book. There was a great need for someone equally versed in biology and psychology to write a book such as *Programs of the Brain*, and Professor Young has succeeded in getting the essential logical points across to the non-specialist reader. There will be no excuse, after reading this book, for confusing the proposition that innate factors control psychological development with the preformationist concept of 'innate ideas'. Equally, it is made clear that 'learning' cannot be invoked in complete opposition to nativism, as learning itself is nothing but a program for acquiring certain kinds of knowledge. Stones, after all, learn nothing, and different animals learn quite different things. This reworking of the nativist–empiricist controversy has been commonplace in ethology for some year now, but it is useful to have it so clearly brought to the attention of the general reader.

Logical clarity alone, however, does not tell us how things work. And the unsatisfactory feature of this book, as J. Z. Young freely admits on several occasions, is that we really don't know how 'programs of the brain' work at all. The problem with a metaphor such as 'program' is that it gives an illusion of understanding, an illusion that gains powerful force from mere repetition. A previous generation of psychologists was excited by the telephone exchange model of the brain. The computer analogy is very probably an improvement, but as this book concludes with proper detachment, in the future ". . . perhaps a new form of laughter will also echo through the system, as the information comes up that in 1978 the best way the ancients had to describe these processes was to call them Programs of the Brain". One does therefore wonder what is to be gained by saying that there are programs for "loving and caring, fearing, hating and fighting, hearing, speaking and writing" (to mention but a few from the chapter headings). We are given very little clue as to how these programs work, and the mere verbal redescription smacks uncomfortably of the behaviourist's irritating habit of relabelling something like play as "ludic behaviour" and thinking that they had said something substantial.

One interesting hint J. Z. Young throws out is that brain programs for vision may depend crucially on topographical representation of the input. He suggests that our capacity for solving problems by visual imagery may depend on cortical maps of this sort. It would be interesting to have a further working out of this idea, which—understandably in a book of this scope—is not expressed in anything more than an allusive form. The idea seems to be that visual representation may consist at least in part of 'symbols', which in themselves have some features of the represented state of affairs,

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rather than being mere arbitrary 'signs'. This useful distinction between signs and symbols, which J. Z. Young makes rather clearly at one point, is unfortunately blurred later on when he speaks of colours as 'symbols'. The more usual view is that colours are arbitrary signs for different wavelengths of light. When J. Muller gave the first systematic expression to the doctrine of specific nerve energies in 1838 (not 1848 as stated in this book) he was expressing the idea, well known to Hobbes and other philosophers, that the quality of a sensation depends on the nerve that is stimulated, rather than on some property of the stimulating agent. Young and Helmholtz later extended this to different nerve fibres, proposing that perceived colour depends on the nerve channel stimulated. This seems to make colour a purely arbitrary sign. But J. Z. Young is perhaps insisting on something more profound in calling colour a symbol. There would be no need for an animal to respond to a wavelength at all unless it were significant in some biological sense, and it could be that colour is a symbol of this significance, as the phenomenologists such as Merleau-Ponty have argued, instead of being an arbitrary sign of a wavelength.

As this book is written for the non-specialist it is worth noting with satisfaction that it has been produced by the Oxford University Press for only £5.95, complete with many excellent illustrations. This excuses certain oversights, such as a systematic disordering in the page references to the Bibliography, as if the latter had been added to at some stage without corresponding changes in the index. When *Programs of the Brain* goes into further editions, as it undoubtedly deserves, one hopes that this minor imperfection will be put right. □

M. J. Morgan is Professor and Head of the Department of Psychology at the University of Durham, UK.

Gloss on scientific practice

Stuart Sutherland

How We Know: An Exploration of the Scientific Process. By M. Goldstein and I. F. Goldstein. Pp.357. (Plenum: New York and London, 1978.) $17.94.

IT is a truism that the finished product of scientific endeavour—the successful theory—is very different from the halting steps by which it is usually reached. *How We Know* attempts to describe both the methods of discovery and the product: it is more successful at characterising the former than the latter.

Half the book is devoted to three illustrative case-histories—Snow's proof that cholera can be transmitted through drinking water, Rumford's experiments on heat, and the history of research into schizophrenia.

Snow is often credited with being the founder of epidemiology and although his work is well known, the story is worth retelling in somewhat more detail than usual. The final proof of his hypothesis was based on his discovery that one district in London was supplied by two separate water companies, one of which drew its water from a comparatively uncontaminated stretch of the Thames, the other from a region containing much of the sewage of London. Snow was able to show that although the houses supplied by the two companies were quite randomly distributed within the district, the death rate from cholera was ten times as high in the houses supplied by one company as in those supplied by the other. This finding was the culmination of much patient work. Snow already knew, for example, that doctors, who always washed after attending a patient, rarely caught cholera, whereas those "women of the working class" who laid out the bodies and made "the occasion one of eating and drinking" frequently did. Having formed the hypothesis that cholera was transmitted by swallowing "the ejections and dejections of cholera patients", Snow persisted with it in the face of some evidence that seemed incompatible. Not everyone who drank heavily contaminated water became ill: the same argument is still used by confirmed smokers as a disproof of the fact that smoking causes lung cancer.

Both the account of Snow and that of Rumford gain in interest by being told largely in their own measured and elegant prose: if only today's scientists had the talent and leisure to write like their predecessors, how much more pleasureable the pages of *Nature* would be. Like Snow, Rumford persisted in clinging to a hypothesis: he believed in the kinetic theory of heat at a time when the caloric theory—that heat is a substance—was widely accepted. He showed that within the limits of his apparatus heat had no weight and that when cannon muzzles are bored an indefinite quantity of heat could be produced by friction; he also discovered molecular diffusion, attributing it to the motion of molecules. But although his theory was correct, there were too many facts that at the time seemed more consistent with the caloric theory for it to be accepted: the rival theory gave a better account of such phenomena as latent heat and the transmission of heat by radiation across a vacuum. As Martin and Inge Goldstein note, the caloric theory, although wrong, itself led to many discoveries, including the laws governing heat capacity and the transmission of heat from one substance to another.

The authors' account of schizophrenia is perhaps the least interesting of their three case-histories: our knowledge of schizophrenia has been advanced by many different investigators and the story does not bear the imprint of a single mind in all its individuality. Moreover, as they acknowledge, they omit a great deal of the evidence that bears on the nature of schizophrenia so that some of the conclusions they record stand up rather better than is evident from their description. For example, they do not mention the World Health Organisation's massive study which established that both the prevalence of the illness (at least in its 'nuclear' form) and its symptoms show little variation across nine widely divergent cultures.

Although their case-histories make agreeable reading, the conclusions the authors draw from them are hardly new. Scientific discovery usually stems from the more or less systematic testing of hypotheses; luck may play a role; the investigator must be ready to take advantage of the chance observation that may prove significant; intuition plays a large part; the mechanisms underlying creativity are unconscious and ill understood; and stubborn persistence in a hypothesis in the face of apparently contradictory evidence can lead to major advances. They forebear to point out that persistence can also lead to disaster—any scientist knows of colleagues who spend dismal years following out an unprofitable hypothesis or approach: it is impossible either to know in advance whether persistence will be rewarded or to characterise the intuitions which enable the best scientists to judge when persistence is warranted and when it is not.

On the progress of science, the Goldsteins take a Kuhnian position, but like him they tend to overdramatise the nature of revolutionary changes in scientific paradigms. It is true that in some cases, such as Newtonian physics, the theory of evolution, or relativity theory, a single piece of work has made a great many seemingly disparate facts fit together and has at the same time produced a fundamentally new way of looking at things. But even in

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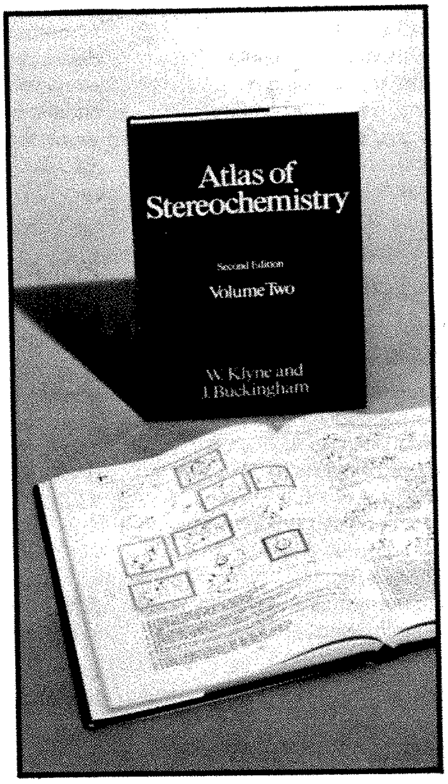

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such instances many of the ideas were in the air at the time, and in other cases revolutions in ways of thought have occurred more insidiously. Pavlov and Sherrington, for example, had the fundamental insight that it was possible to investigate the nervous system by examining its input and output and inferring the intervening mechanisms, but much of Pavlov's own theorising was wrong and despite the importance of his research, Sherrington's *magum opus, The Integrative Action of the Nervous System* did not itself integrate many facts. Moreover, the Goldsteins place little emphasis on the importance of new techniques, the development of which often leads to fundamental new insights.

Their discussion of the finished product of science is inadequate. They point out that the Azande's beliefs about witchcraft are internally consistent and impossible to refute either by appeals to common sense or logic, and elsewhere they note the similarities but not the differences beween scientific and artistic endeavour. They raise but do not answer the question "Is what we have called the scientific method . . . a better, surer road to truth and understanding than any other?" Partly through Kuhn's influence, and partly perhaps through a loss of confidence in our own civilisation, it has become fashionable to lump science together with other systems of thought and to claim that it has no unique claim to objective truth: this tendency has surely gone too far.

Mystical systems do not enable us to control or to predict accurately events in the outside world though some may help us to control ourselves; most such systems could be as tenaciously held as they are if the world were very different from the way it is; science is not just one amongst many possible and equally valid ways of viewing the world. It attempts to construct models which fit the facts with the utmost precision, and which combine generality with economy. Works of art operate upon us obliquely, are often ambiguous and leave so much unstated that different people can project into them different significances. Scientific theories, on the other hand, attempt to state unequivocally all their underlying assumptions, to remove all ambiguity, and to arrive at a system that can only be understood and manipulated in one way.

Despite its failure to come to terms with the nature of the products of science, *How We Know* is an interesting, if slightly elementary, gloss on scientific practice. ☐

Stuart Sutherland is Professor of Experimental Psychology at the University of Sussex, UK.

Dynamic equilibrium of science

John Ziman

Science as a Human Endeavour. By G. F. Kneller. Pp.333. (Columbia University Press: New York, 1978.) $17.45.

SCIENCE used to seem simple. There were Laws of Nature to discover. There was a Scientific Method. Scientists were clever people, although aloof and uncultured. The marvellous new devices that benefited mankind came with dangerous new ideas that sapped the consolations of religion. Science was inhuman and incomprehensible; it was fortunately useful, even if it was unfortunately true.

To the more realistic studies of recent years, science has begun to seem more and more diverse, and more and more paradoxical. Its subject matter can vary from butterflies upon the wing to neutrinos trapped in a black hole. Methods of research range over techniques as diverse as electronic data-processing, opinion surveying, cinephotography and the playing of simulation games. An experiment may involve launching a space probe to Venus—or persuading a student of psychology that he is (perhaps?) near to electrocuting an innocent victim. A theory may be all algebra, or all words—and it may apply to the symmetry group of an elementary particle or to the elementary behaviour of a small human group. Scientists themselves vary from the speculatively divergent to the pedantically convergent, from sober realists to wild fantasists, from saints to sinners, from the fastest wits to the slowest bores, from high achievers to utter bunglers.

In the making of science there is the ever-present contrast between the solidity of established knowledge and the fluidity of conjectured novelty. In its applications, the incalculable benefit of a penicillin cannot be squared with the calculated horror of a napalm. Scientists belong to the most universal, transnational world community; at the same time, they are the most trusted servants of their warring governments. Scientific knowledge is as objective as we can make it; yet it is created out of the most subjective sweat of the individual human brow.

This diversity out to the limits of conceptual, technical, psychological, political and moral contradiction, characterises science as a human activity. It cannot be cramped into narrower formulae, such as "All science is quantitative", or "All science derives from technology", or "All science is revolutionary" or "All science must serve the State". It would be a profound error to recoil from the evident complexity into yet another over-simplified stereotype.

With this, I think, Dr Kneller would agree. His excellent chapter on "The Scientist as a Person" emphasises the historical diversity of personalities and styles of thought. But he has not faced up to the necessity of finding the unifying factors in this vast endeavour. There is more coherence than he discloses, in the philosophical analysis of competing research programmes, the sociological analysis of norms and invisible colleges, or the economic analysis of science policy. He has read about, and adequately summarises, these various manifestations of the contemporary study of science, but fails to capture the leviathan beneath the surface. This is a graver defect in a thoughtful and lucidly written book than the many minor errors of fact that would irritate a well informed reader.

The heart of the scientific enterprise is the dynamic bond between individual and collective forces. Personal attributes of curiosity, intelligence, creativity and ambition are cleverly harnessed by communal restraints of organised criticism, institutionalised competition and the kicks and halfpence of social esteem. The "search for order in nature", Popperian falsification, Kuhnian paradigms, referees and peer review, graduate education, and many other diverse and apparently contradictory features of science, fall into place around this tense and quivering bond. This is the secret of the "Scientific Method".

What comes out of this is a body of knowledge with a few general characteristics. To achieve an adequate degree of consensus, science must concentrate on the material, demonstrable, publicly shared aspects of the world. The product of long, deep and highly imaginative study may look as 'unreal' as quantum theory; but this is no more mystical or unpractical than the mechanism of an earthquake or the anatomy of a louse. Ultimately a scientific theory can only commend itself by its predictive power—by an evident coherence between a conceptual scheme and the results of deliberate action.

Because it has had to stand up openly to expert criticism, established scientific knowledge is reasonably free from wild fantasies and obvious inconsistencies. But the product of fallible human beings inevitably contains many minor mistakes, as well as grand misconceptions derived from the general opinions of the day. These deficiencies must be allowed for—yet they are incorrigible by reference to any principle that lies outside science. Scientific error can only be driven out by further science. Such progress may be much slower and more difficult than we would like—and in some cases may even prove illusory. Yet the process of self-correction is inherent in the method of science.

Scientific knowledge, being active rather than contemplative, is eminently applicable. Sophisticated technology may derive in large measure from science, and may provide the means and the motives for the investigation of particular questions. But neither the needs of practice, nor human aspiration, can determine the outcome in advance. Scientific knowledge is as objective and neutral as a frying pan or a sword: it is fit for such uses as those who possess it may desire, and for which it is appropriate, but neither can cook nor kill on its own account.

For all the philosophy and political sociology in the world, these maxims cannot be seriously controverted. But the scientific endeavour is questionable in several aspects that are scarcely mentioned by Dr Kneller. What is the potential scope of the scientific "method"? We feel sure that it cannot encompass ethical or aesthetic issues. When allowance has been made for the reflexive feedback of consciousness, can science provide reliable knowledge about human behaviour in general? Is the objective of a 'science of society' essentially self-contradictory—a bootstrap exercise in which one always ends up flat on ones back? This question is important, because every unjustifiable claim to scientific expertise in social affairs is a terrible danger to humanity.

Another serious question is whether the social system of science can stand the strain that is put upon it by modern society. The scientists of our day still live and work according to the principles of the academic science of the past. But will the dynamic equilibrium between individual and collective forces still be possible in the industrialised and bureaucratised technical organisations of present-day research? The fundamental contradiction of contemporary science is between the technocratic impulse to turn science into a pliable instrument of policy and the pluralistic view that it must be allowed to go its own way as a fertile but erratic source of potentialities for understanding and action. There is a real risk that in our greed for technological golden eggs we shall try to put this wild goose into a battery and kill it with too much rich food. For three centuries, science has been a magnificent human endeavour; can it survive its own success? □

John Ziman is Professor of Physics at the University of Bristol, UK, and Chairman of the Council for Science and Society.

Limits to scientific growth

F. S. Dainton

Scientific Progress: A Philosophical Essay on the Economics of Research in Natural Science. By N. Rescher. Pp.278. (Basil Blackwell: Oxford, 1978.) £15.

As an undergraduate reading chemistry I found in the College Library a remarkable book by R. G. Collingwood called *Speculum Mentis*. The clarity and cogency of the argument and prose style almost seduced me from chemistry to philosophy. From this temptation I was saved by reading Hishelwood's *Kinetics of Chemical Change* which convinced me that to be a scientific specialist need not exclude other concerns. Why should scientists not have something to say about philosophy and philosophers something to say about science from which their auditors could profit? Now, 46 years later, I recall with pleasure the enlargement of my understanding gained from reading not only the works of past giants but also the works on science and philosophy of many near-contemporaries like Medawar, Popper and Polanyi. I return repeatedly to peruse them and always with profit. Alas, I am also reminded of the growing volume of mediocre work purporting to deal with the philosophy of science, and science policy. Much of these writings obfuscate rather than clarify; their prose is loaded with impenetrable, imprecise jargon—the hallmark of the institutionalisation and decay of an intellectual enquiry—and I put them down never to return to them.

This book does not fit easily into either of these categories. The author is prolix to a degree; printing costs hold no terror for him and if 11 words can be replaced by 4 he is unerringly generous. Thus, for "Figure X illustrates this" he writes "the case is rendered graphic by the picture of figure x" and for "After phase one . . ." we read "once the first phase has been gotten over with . . .". More significantly the book is stuffed with lengthy quotations and with tedious tables and graphs telling the readers what most of them know, namely, that scientific activity, however measured (for example, by numbers of scientists, by their publications, by their costs, by growth of techniques, and so on) has grown phenomenally in the past two centuries.

All this verbage obscures the message which is simple and could be encompassed in one-fifth the space with five times the impact on the reader. The proposition is that scientific progress is not limited either by any intrinsic finiteness or by human cognitive power, but that, although knowledge begets knowledge, which accounts for the near exponential progress of science in the early stages, scientific advances now and in the foreseeable future require even more effort and therefore the deployment of more human, material and correspondingly financial resources. Consequently acceleration gives way to deceleration of scientific advances and the smoothed (for the author acknowledges the episodic nature of discovery on the short timescale) curve of scientific progress over centuries will appear sigmoid. This is not to say that there is an asymptote which is constant so much as having a slight positive slope.

Two features of this conclusion, which I can accept as probably correct though unprovable, are puzzling. First, to whom is the conclusion addressed and what use should be made of it? Governments, their agencies and large private and national corporations who provide the bulk of the money for scientific research already know that it becomes daily more expensive in real terms. This fact rather than the argument of this book is what sharpens their choices and affects their priorities. Will it help the scientists, stimulate their endeavours, quench their enthusiasm or send them off in new directions? I do not know but I doubt it.

What will the philosophers make of the book? I am not competent to express a view as to its philosophical significance though I would guess that it would have a value in slightly deepening their perceptions of the extrinsic factors which now restrain the rate of advance of science but which leave their own subjects virtually untrammelled. Nor will the economists or philosophers of science bat an eyelid for there is no news for them in this book. But it would make useful background material for school teachers, whether of history or of science, and

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for undergraduates in the liberal arts and science colleges.

The second puzzling feature is that although the author refers to human will as influencing the pace of scientific progress he scarcely touches the more fundamental question as to what potentiates this will, though this is clear enough.

Science like art, music and literature is one of the ways in which man learns more of his own nature especially in relation to the external physical and biological world. The pursuit of science is part of the quest for his own identity. Additionally, scientific knowledge gives him power to use the forces of nature for his own ends and may even change some of the values by which he lives. What seems to me to be important here is whether Western man, whom science has enabled to meet his biophysical needs will opt in the post-industrial society to satisfy his psychosocial needs and what effect this will have on his will to advance science. Even if, as Daniel Bell has indicated, the optional post-industrial society is the learning society, will man want to know more of Nature or more of the nature of Man?

Although I have described the main thesis it should be made clear that in developing it the author refers to other interesting matters. Thus, the importance of technology, even its necessity, for the advance of science is clearly delineated, although the almost inadvertent way in which science can provide the seed of much technology is scarcely developed. There are provoking juxtapositions such as the increasing cost of pollution and the diminishing cost of computers, attempts to provide a taxonomy of subjects and of technological speciation. But even giving credit for these virtues the book is not remotely worth £15 for which price one is entitled to expect the absence of elementary spelling errors such as *polution Wertheimer* (the author of one of 3 "magisterial" reports upon which "the book can be regarded as a philosophic—economic commentary") and George *Babbington* Macaulay whose second page reference in the index I could not find. ☐

Sir Frederick Dainton is Chairman of the University Grants Committee, London, UK.

Universalistic view of science

Stuart Blume

The Reward System in British and American Science. By J. Gaston. Pp.204. (Wiley: New York and Chichester, UK, 1978.) £13.40.

THE most important criticism which can be levelled at Professor Gaston's highly polemical work is not that the arguments it contains are in error (though in some ways I believe them to be) but that it embodies a minimising and depressing answer to the question "what should the sociology of science be about?" No scientist, whether physicist or sociologist, wants his specialty to be defined so as to exclude the questions he finds most interesting. Let me explain, though necessarily at some length.

Some 20 years ago the distinguished American sociologist Robert Merton set out certain rules, or 'norms', of social behaviour to which he believed scientists tended to conform. These norms, a sort of moral code, were seen as uniquely congruent with the goal of science: that is, the advancement of knowledge. (For example, one such norm was 'universalism': the notion that any contribution to science must be judged solely on its scholarly merits, and independently of the nationality, sex, institutional affiliation or age of its author.) Conformity was secured by rewarding 'proper' scientific achievement, both formally (with medals, invitations to serve on this or that) and informally (by citation of work): symbols that scientists came to want. Out of Merton's observations grew a sociological research programme, central to which was investigation of the reward system of science.

Either by personal interview or with a mailed questionnaire, data were collected from groups of scientists. Indicators of each individual's contribution to science on the one hand, and the 'rewarding' or 'recognition' received on the other, were correlated. The point was to see whether such extraneous factors as where the scientist had been trained, or where he worked, influenced the reception his work received, independently of its quality. Some of these studies (including that by Sinclair and myself of chemists in British universities) indeed found such "departures from universalism". (We found that an Oxbridge affiliation (a post at Oxford or Cambridge University) was an advantage in chemistry.)

After reviewing some of these studies, Gaston disputes the interpretation which the authors placed on their findings. His view is that systematic departure from universalistic behaviour is unproven. Moreover, in so far as there is any truth in the previous authors' claims, what is indicated is that the reward system works differently in the various disciplinary and national groups studied.

In the central part of the book (pp55–119) Gaston sets out to see if this is true. Information on a random sample of 600 academic scientists (divided equally between physics, chemistry and biology, and between Britain and the USA) was collected from published sources such as *Chemical Abstracts* and *American Men and Women of Science.* The two countries are seen as exemplifying contrasting styles of organising and funding research, and the three disciplines different levels of "codification" (=development). (For reasons of space I won't take issue with the crude reductionism underlying this view of the relationships between the sciences, nor indeed with the somewhat misleading picture of the British educational and science policy systems presented.) Analysis takes the form of many pages of correlations and regressions, involving such variables as each scientist's number of publications, years of research, 'honours' received, citations to his work, and so on. In brief, the conclusion is as follows. Factors such as sex or the prestige of the university of training or employment make little difference in science. Scientists are honoured, their work cited, almost wholly according to merit: the system is "universalistic". Not wholly so, however, and the differences between the disciplines turn out to be greater than those between the two countries.

Gaston's major conclusion, that science is largely universalistic, implies that science is unique in the modern world: unique not just epistemologically but sociologically too If race, sex, social origins, place of work are irrelevant to the working of the scientific system, this is scarcely true of any other sphere of modern life. The claim that science is universalistic implies (though this is not examined) that scientists' commitments and loyalties, all the things that they are and that concern them when they leave the laboratory, are irrelevant to their systematic interactions with each other. In Britain a public school or Oxbridge education may be an advantage to the physician, the civil servant or the soldier—but not to the scientist. That may seem reasonable, but one can go on. Does it seem as reasonable to hold that to be invited to serve on a top-level advisory committee depends solely on scientific achievement, and not at all on politics or whether someone works in Massachusetts or Wyoming? Women are said to be at no disadvantage in science, and the fact that the sample of 600 included only 3.7% women is nothing to do with science. As this universalism is said to be almost independent of political and organisational contexts of science, it should be

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hard to conceive of a country in which the honouring of a scientist's work could be influenced by any dissident views on political matters which he or she may hold.

It is a fallacy to believe that people's concerns, loyalties and commitments are so rigorously compartmentalised: that the fact of parenthood is irrelevant outside the home, or of political commitment outside organised politics. If these things are indeed irrelevant in science, then surely those who hold this to be so are by now obliged to prove how and why this is and remains so. The onus of proof must be shifted.

The divergence of view between Jerry Gaston and I derives in part from our different methodological preferences. What is to count as evidence? Should we correlate membership of the Royal Society, the US National Academy of Sciences or the Soviet Academy of Sciences with "number of papers published" — or should we try to probe how, and on what basis, these bodies actually elect members?

We differ also on what we want our subject to be about. On page 14 it is stated "Scientists cannot be totally motivated to conduct research by the level of monetary rewards". But are they totally unconcerned? Is the growth of trades unionism among scientists wholly irrelevant (to sociologists)?

The depressing thing for me, then, is the implication which the book embodies as to what the sociology of science is to be about. If the subject is about the operation of the "reward system in science" — indeed central to the research tradition which Gaston so proudly espouses — and if this works virtually independently of socio-political environment, then what should sociologists of science do? There is little point in studying the way the system works in a traditional society like India or a culturally divided one like Canada. Yet interesting work of this kind is being done. There is little place in such a sociology for studies of the way in which intellectual and social changes in a discipline interact with each other over time. Yet here, too, fascinating work is being done by sociologists, historians and philosophers working together. A crucial test of any research programme must be the range and the interest of the problems for research which it generates. However convincing his statistical correlations, on this test Professor Gaston's work falls. ☐

Stuart Blume is Senior Research Fellow in the Department of Sociology and Psychology at Chelsea College, University of London, UK.

Lifeboat or country club?

Thomas H. Jukes

The Limits of Altruism: An Ecologist's View of Survival. By Garrett Hardin. Pp.154. (Indiana University Press: Bloomington, Indiana, and London, 1978.) $10.

ALTRUISM is a fascinating and inexhaustible topic for writers. The betrayal of kindness by ingratitude is a recurrent and compelling theme in drama. Throughout human affairs there runs a curious and shifting equilibrium between selfishness and generosity, but, as Hardin notes (p79) starvation puts an end to altruism. Human actions divide into opposing categories of egoistic and altruistic.

For some years, Hardin has discussed problems of exhaustible resources, often in terms of analogies. One of the themes is that of a shared pasture that becomes irreversibly depleted by overloading with individually owned grazing animals. This is well illustrated by the current international depletion of oceanic fisheries. In *Living on a Lifeboat* (p142), Hardin says that "To avoid unconscious suicide, we are well-advised to pit one metaphor against another". His lifeboat metaphor, which surfaces again in *The Limits of Altruism* (p62) says that it is foolish for the well-to-do nations, living in lifeboats, to go on welcoming members of the impoverished nations aboard. The lifeboat will sink with all hands.

However, I think that inhabitants of the impoverished countries are apt to counter with another metaphor. The prosperous nations remind them of the wealthy members of a country club who have closed the doors to outsiders because the golf course and the dining room might become crowded. Does anyone believe that we in the developed countries can keep the swimmers around us from climbing aboard the lifeboat—or from overturning it? Hardin evidently believes this. He says that we should "preserve enough of the economic and social barriers between groupings of humanity so that the cancer of collapse can be localised" (p84).

He states with emphasis that "Selection is not, by its nature, for the good of the species. The survival of the species is an almost accidental by-product of the survival of germ lines" (p107). However, it is obvious that germ lines cannot survive if the species becomes extinct, so therefore survival of the species cannot be an "almost accidental by-product".

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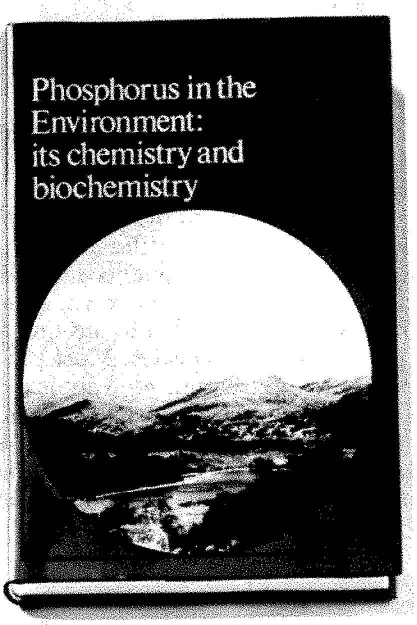
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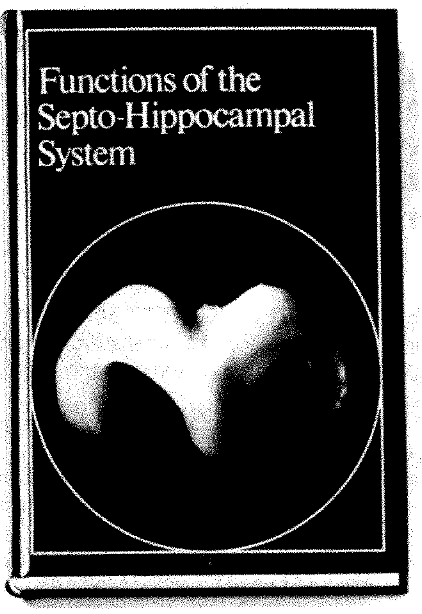

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In our species, says Hardin, selection occurred on a tribal basis. Altruism was practised within the tribe, and non-members of the tribe were exterminated (p112). Yet today (p127) "we continue to fling money desperately at poor countries half a world away, hoping for miracles" . . . "If we desire a world in which altruism can persist, we must reject the ideal of One World . . ." (p132). Whether such a rejection is feasible is not clear. ☐

Thomas H. Jukes is Professor in Residence, Division of Medical Physics, University of California, Berkeley, California.

Early Victorian science

David Knight

Science in Culture: The Early Victorian Period. By S. F. Cannon. Pp. 296. (Neale Watson: New York; Dawson: Folkestone, UK, 1978.) $17.95; £12.50.

It was with a plea for history in depth, published in 1964 and revised as a chapter of this book, that Dr Cannon made a reputation; as befitted the journal *History of Science* in which it appeared, this was partly a review of recent work, partly a discourse on method, and partly an elucidation of an historical event. The various chapters of this book are all of rather the same kind, several others being revisions of articles; together they form a rather curious and loosely organised but very stimulating book, which poses more questions than it answers. Some chapters are very informal, and read like lively contributions to a seminar; thus, on one page "Thomas Kuhn" is introduced in one paragraph, and becomes successively "Thomas" and "Tom" in those that follow it. In sundry asides, we learn something about the author; for example, *apropos* of a discussion of professional success: "It is nicer to sit at the top of a new multi-million dollar building in a world-famous institution and let the seekers come to you. This is what I do". The founders of the British Association might perhaps have felt something like that, though they would have blushed to write it.

Dr Cannon's desirable office is at the Smithsonian, and one might expect that museums would occupy a prominent place in her discussion of early Victorian science; but they do not. In general, botany and zoology, and the world portrayed in D. E. Allen's *The Naturalist in Britain* (1976) get short shrift; also omitted is discussion of medicine, in which men of science (even physical science) were trained and which was undergoing professional reorganisation during Dr Cannon's period. Nor is chemistry seriously discussed, although it could have been, as Colin Russell *et al.* have shown in their *Chemists by Profession* (Open University Press, 1977). Morris Berman, in his *Social Change and Scientific Organisation* (Heinemann, 1978) presents the Royal Institution as a temple wherein Day and then Faraday, apostles of applied science, preached to improving landowners; but applied science is not one of Dr Cannon's interests, and the reader will find little to remind him that Britain was in the throes of industrialisation, or that important educational centres were being founded in London and the provinces.

Dr Cannon is exposed, as anybody who advocates the ideal of history in depth must be, to anybody who cares to point to what she has left undone; but if the historian really had to take everything into account before he or she wrote anything, then the discipline would wither away. So it is within its limitations, as an account of what she calls the "Cambridge Network", that the book should be judged; and there it is illuminating. This is upper-crust history; her protagonists share an admiration for Broad Church doctrine and physical astronomy, and are professionals in the lofty sense that they pose the questions which the ordinary cultivators of science then try to answer. This view of professionalisation gives one a moderately testable version of Kuhn's idea of paradigms, and Dr Cannon's discussion, in general terms and applied to the founding of the British Association, is most interesting. John Herschel is particularly important in her study, and she makes out a good case for her remark that to agree with him "was considered a mark of good sense in early Victorian England".

For her, Herschel and his associates in the "network'—of which Faraday was a kind of honorary member—played a vital role in bringing to birth what we call 'physics' out of natural philosophy by Humboldtian observational science. She admits in a note that this is a partial view, and that steam engines had something to do with it too, but her part of the story is fascinating, and here as elsewhere hoary myths are elegantly shown up. Her suggestion of most general interest is that up to the 1850s in Britain there was a single view of truth, with science, religion, literature and history being complementary, and Newtonian astronomy the accepted model of demonstrated truth. She sees this golden age

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or era of good feeling collapsing in 1859 when Darwin published the *Origin of Species*, and not merely two but a whole series of cultures emerged, each with its own experts, or professionals, and tests of truth. This is interesting, and may be true; but if so, it would only show that events in England moved more slowly than they had in France or Germany, where the process had begun much earlier. One may also suspect that a focus on her Cambridge men—a group dedicated to the unity of knowledge—makes one attribute too much importance to them, and especially to Darwin, who appears here as the only star of any magnitude in the life sciences; Huxley comes in as his accompanist, rather than as the introducer of the revolutionary new physiology from the Continent. In the decade before 1859, religious doubt had spread widely in Britain, and novels describing it had become a *genre*; men such as Frank Newman and J. A. Froude were prominent agnostics owing little or nothing to Darwin or his circle.

One may suspect then that some of Dr Cannon's generalisations apply not far beyond the limits of her "network"; but like the professionals she describes, she poses questions that will set others thinking for some time (and indeed have already done so) and she makes methodological remarks of great value to anybody interested in the history of science. □

David Knight is Senior Lecturer in the History of Science at the University of Durham, UK.

Foster's school

R. K. French

Michael Foster and the Cambridge School of Physiology: The Scientific Enterprise in Late Victorian Society. By G. L. Geison. Pp.401 (Princeton University Press; Princeton, New Jersey, 1978.) £18.40.

HERE is a biography of Foster and an account of the Cambridge School of Physiology. It is not, as the subtitle suggests, about *The Scientific Enterprise in Late Victorian Society* as it is limited to "one small group of English physiologists . . . between 1870 and 1900" (preface).

Although we are not far removed in time from late Victorian science, there is still a great deal we do not know about some of it, including physiology: Geison's book is a step in the right direction. Modern fashions in history range from history of ideas to sociological history, and historians, if not the readers of historical books, look for more than the old-fashioned Life and Times biography. But only rarely have we enough information to make the most of both of these approaches.

The format of this book is determined partly by the demands of social history, but the book itself is something of a pilot study in an area which, as Geison insists, is still very little known. The result is that these modern historical tools cannot be used effectively, and the text gives the impression of being somewhat over-written, a little bit 'stretched'. Another reason for this is the book's origin as a dissertation.

The copious text is subdivided by elaborate headings, which suggest a somewhat laboured reworking of the material. The reader might even be well advised to read first the "Concluding Reflections" which so lucidly and tersely tie together the strands of the book that they are an indispensable guide to the detail and documentation of the earlier pages. These are naturally more exploratory than books written later in life. In his exploration Geison has uncovered a problem with Foster and has made valuable progress in seeing how it is answerable. Foster's reputation is that of a man with great influence on his subject and at the centre of its institutionalisation, yet the further the evidence and explanations for this are pursued the more elusive they become. Perhaps this problem can be historically generalised as the 'Boerhaave problem'. Boerhaave was one of the most renowned medical teachers of all time, yet the reasons for his influence are difficult to pin down. He made no major discovery and his teaching contained few innovations. His successful synthesis of current systems must be part of the answer, but the resultant bland and conservative doctrine does not convince even the historian of ideas that it was the complete answer. Such a historian is compelled to use other techniques.

Geison's problematic Foster faded early as a research physiologist without having produced "a signal piece of research of the first order", and as the quotations prefixed to Geison's introduction show, neither contemporary or later historical opinion could in any concrete terms explain Foster's influence even by reference to his teaching. So, even though the problem calls for new historical techniques, the evidence is insufficient for their use. Geison's attempt to use them is dutiful but not always productive; at one point he is "ready to give up in despair" (p143) in attempting to solve the "mystery of Foster's achievement [which] can never be entirely dispelled" (p188). In attempting to dispel it Geison very usefully investigates Foster's work on the myogenic origin of the heartbeat, his characteristically English evolutionary view of physiology, which form a strong 'history-of-ideas' theme for the book, and his tactics to promote his 'school'—for example his publication of his student's papers. Much more hypothetical is Geison's brief and flirtatory parallel between on the one hand the politics of Bismarckian Germany, Czarist Russia, and the neurogenic 'autocratic' theory of heartbeat, and on the other, the opposing, innate, non-centralised 'democratic' myogenic theory, and Foster's role therein.

Readers of *Nature* will probably find these remarks too carping, and will certainly find the book itself a very welcome piece of work, carefully researched, dealing with an important topic and necessarily interesting to modern scientists and historians. □

R. K. French is Director of the Wellcome Unit for the History of Medicine, University of Cambridge, UK.

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Fragments of Chargaff

Jerry Donohue

Heraclitean Fire: Sketches from a Life Before Nature. By Erwin Chargaff. Pp.252. (Rockefeller University Press: New York, 1978.) $13.

IT seems appropriate to set the stage of this review with a quotation by the author, one which gives a bit of insight into the character of the character that will be dealt with:

> "I cannot but find it remarkable that almost all the recognition my work received has come from Europe".

Why does he, full professor and department chairman at Columbia, awarded two Guggenheim fellowships, elected to the US Academy of Sciences, recipient of the National Medal of Science (and that's no *petite pomme de terre*) think this? Perusal of the *Citation Index* for the years 1965–68, to choose a few years at random, shows three or more columns of entries for all four years, from predominantly American journals—is not citation recognition? Then why, in 1970, did he submit an important essay, in German, to an obscure German monthly, and eventually publish it in not quite so obscure Swiss journal? This same essay was later translated into English, published in *Science* (**172**, 639; 1971), and in this form "became, perhaps, the most widely read of my articles."

This book was easier to read than to review. The first time I read it, I, well, detested it, and the further into it that I ploughed, the more I was distressed by the author's naïvité, self-pity, anti-Americanism, propensity to quote in foreign languages, inveterate name-dropping, recondite vocabulary, and much more. However, the second time around I found myself trapped into admiration of his observations on: Science (the field, not the weekly tabloid), scientists (some of whom, alas, are only alluded to and not named), Columbia University, molecular biology, current research conditions, DNA, and many more, as well as his piercing personal opinions on almost everything. But more of these later.

One especially valuable feature is the inclusion of a name index, with identifications, which runs to nine pages. However, it is unfortunate that not all of the names dropped in the text are indexed. Furthermore: is it really necessary to have such entries as:

> "Carter, Jimmy—US president
> Ford, Gerald R.—former US president
> Hitler, Adolf—Austro-German politician
> Michelangelo Buonarroti—Italian artist

'Twould have better to have pruned out the obvious, such as those above, plus a plethora of more, and to have included most of those omitted, such as Biedermeier, Consden, Doniger, Gordon—where and who are they now? Other names found in the text were also unindexed; should not these have been included? for example:

> Khan, Genghis—Mongolian politician and explorer
> Terrible, Ivan the—developer of Siberian real estate
> God—Supreme Deity

Because of the fragmentary nature of this book, I decided to cast this review in the form of numerous excerpts, letting the author largely write his own review, so to speak.

Language, and use thereof

Chargaff has a decided tilt towards quoting titles, phrases, sentences, and long (sometimes very long) paragraphs in foreign languages. These are often, but not always accompanied by translations, some of which are unnecessary, for example, *Radetzkymarsch*= Radetzky March. (This may be the fault of his copy editor, that bane of authors who wish to write in their own style.) I noted passages in German (his favourite), French, Latin, Russian, Greek, Danish, Spanish, and Italian; Chinese is conspicuous by its absence, as are Finnish, Hungarian, and Romansch. His vocabulary in English is nothing less than esoterically florid. Consider the following words: aleatory, antinomy, avernal, biopoiesis, bourdon, chiliast, epigonic, hieratic, maieutic, mephitic, phalansteries, polymathic (I stopped at one dozen). If these send you scurrying to your Webster, join me at the bottom of the class.

Whether he is fluent in all of the above tongues I do not know. He admonishes:

> "Reading Ronsard [French poet] or Goethe [German writer] or Blake [British poet and artist] in translation amounts to listening to a transcription of the B Minor Mass [by Bach, German composer] for the ocarina."

He refers to K. Kraus,

> "probably the wittiest writer in the German language": "He considered language as the mirror of the human soul, and its misuse as a forerunner of black and evil deeds. A grammatical haruspex, as it were he read the barbarous and bloody times to come in the entrails of the daily press."

Totally charming.

And who cannot be engulfed by such liquid prose as:

> "I tell what I am told. Who is the speaker? If it is memory, then why does it sometimes whisper, sometimes shout, often chatter, and mostly remain in sullen silence? Ancient telephone numbers from my parents' apartment in Vienna are followed incoherently by the timid half-smile on a little girl's face, and I am eight. Busy ghosts with briefcases run through corridors that no longer lead anywhere. Blind mirrors reflect frightened faces; a bright-red satanic mill engorges bodies and excretes packages of phosphates; gold teeth are sorted, numbered, and melted down; all this to the accompaniment of the sweet and labyrinthine music of the fourth act of *Nozze di Figaro*. It is all in pieces, but the broken edges cut, and there is blood everywhere."

But then we find this sentence, also referring to K. Kraus:

> "The syntactic cataract of his periods, delivered in a specially pointed voice, was an ambush, hiding associations of a shockingly unexpected immediacy."

Now what on earth does that mean?

Also mysterious is his characterisation of the untranslatable (he says) German word *Greis* as

> "so expressive in its diphthongal despair." (?)

On scientists

> "A few years ago, I reviewed a scientific autobiography, a shallow best-seller. In my article [unfortunately not included in the bibliography, although at least one book review *is* included, so the identity of the 'shallow best-seller' can only be guessed at] I had something to say about this species of book, and this I should like to repeat here . . .".

We read that he finds these

> "a most awkward literary genre," [that] "most scientific autobiographies that I have seen give me the impression of having been written for the remainder tables of the bookstores," [and that] "scientists write their life's history usually after they have retired from active life, in the solemn moment when they feel that they have not much else to say. This is what makes these books so sad to read: the eagerness has gone; the beaverness remains . . .".

This book has neither. To be anti-anthropomorphic, Chargaff appears as a mongoose, warily circling the cobras of the scientific establishment, feinting, but ready to strike without warning at the numerous vulnerable points, and it all makes very interesting reading.

> "Great scientists are particularly worth listening to when they speak about something of which they know very little; in their own specialty they are usually great and dull."

There are notable exceptions to this, of whom Linus Pauling is perhaps the most notable; Chargaff himself is another, as evidenced by this very readable book, the fact that you must have your Webster at hand notwithstanding.

> "Most people I meet in my or other universities seem to be rejects from IBM. In fact you can talk with them only in triplicate. Slaves or prisoners of NIH or NSF, of Xerox or Beckman—they are really the narrowest, the dullest kinds of experts or specialists; they are essentially molecular podiatrists: people who know all about the fifteenth foot of the centipede."

Passages like this make me wish that there was some way for Chargaff to reach a larger audience than this book will, to get his messages across. Appearing on TV talk shows to plug the book, as the author of a 1968 scientific best-selling partial autobiography did, is not the answer.

> "In general, there are few things as lugubrious as the professional travels of scientists. This has to do with the professionalisation of our lives and with the unstated, unacknowledged, and possibly even subconscious, cultural imperialism of America. Wherever one goes, the same form of pidgin is spoken, the same cocktails, the same indigestible food, the same centrifuges, gradient makers, the same graphs, all superimposable. After some time, all lectures form one confluent blob; all lecturers stumble through the same ten minutes of automatic recitation; all problems are chattered to pieces, and then coalesce again to the same meaningless phrase, something like 'structure and function.' The brutal clatter of a nonsensical machine engaged in the so-called dissemination of scientific information overwhelms all thought and imaginativeness. Diogenes with his lantern, looking for a human being, would be out of place in these assemblies."
>
> "For instance, the National Academy of Sciences [of which Chargaff has been a member since 1965], which is, after all, only a sort of Chamber of Scientific Commerce into which some very funny characters have entered through various backdoors, is widely regarded as the true receptable of wisdom."

It would have been very interesting if Chargaff had appended a list of these very funny characters, together with their backdoors.

Some nuggets on molecular biology

> "One of the obnoxious dogmas to which it [molecular biology] has given rise—the so-called Central Dogma: DNA makes RNA; RNA makes proteins—is no longer valid . . . But the fact that dogmas could be handed down from the mountains [there are mountains in Cambridge?] shows that science had changed disastrously".
>
> "The definition of molecular biology, given there [in the dialogue *Amphisbaena*, a writing of his not included in the bibliography], as 'the practice of biochemistry without a license' has become well known and much quoted."
>
> "One particularly crude sentence graced the beginning of one of the lectures (the italics are mine) [at a small symposium on biological macromolecules in 1961]: 'The essence of the dogma, *so acceptable to our time*, can be briefly summarized by the following diagram: DNA→RNA→Protein.' (It had not been acceptable to me; but then, I am not of 'our time'.) . . . Actually, this particular dogma, on which free-thinkers and believers could agree so joyfully, has worn unusually poorly. Only a few years later, the peddlers flourished by hawking the reverse dogma".
>
> "There are even some confused people who believe that what is now called 'molecular biology' makes up all the science of life."

On Columbia University

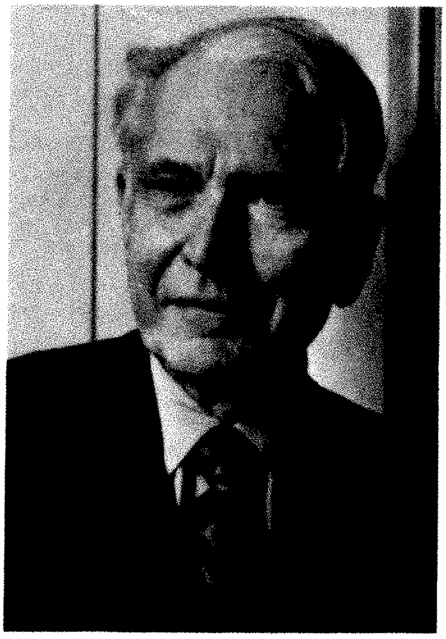
To an outsider, Chargaff's views on the institution where he was on the staff from 1935 until his retirement forty years later, are nothing less than astonishing. Thus:

> "Several [graduate students] already [in 1935] had reached distinction or were soon to do so, although the university showed little recognition of this fact: an old Columbia habit".

And:

> "Many years later, when I occupied the tabouret of biochemistry at Columbia, which in Clarke's time was still a real Chair, I wrote the firm [Eastern Kodak, where Professor Clarke, previous to becoming chairman at Columbia, had spent 14 years developing the imposing line of organic chemicals then and now sold by that firm], suggesting that they help us in setting up a professorship in Clarke's honor. The answer I received will remain a monument to corporate shabbiness".

Although this is less of a blast at Columbia than at Eastman Kodak, the least EK could have done was to have contributed towards a footstool, but that Chargaff chose not to publish that "monument" is regrettable.



Erwin Chargaff

Photo: Rockefeller University Press

And more:

> "What made Columbia, during all my time, such a singularly disagreeable institution is difficult to explain".

Still more:

> "Incidentally, I now have a simple method by which I can recognize future Columbia vice-presidents and deans, especially at the medical school: They misuse the adverb 'hopefully'; they 'address a problem'; they speak of 'input' as if they were dealing with a computer; they submit proposals as 'a package,' but if 'a package' is offered to them, they 'don't buy it'; they like to engage in a 'dialogue'—with several hundred people at the same time—but if you answer, they tell you that this is 'only semantics'." [Loud cheers]

Yet more:

> " . . . I was dismayed to find that my pension amounted to less than 30 per cent of my last regular salary".

What kind of an *Elfenbeinturm* was this man living in? Did it never occur to him to ask someone? Granted that university bureaucracies are notoriously uninformative and mushy, it is, after some effort, possible to extract facts from them.

And finally:

> "On November 20, 1975, the movers came. Certain things had to be left behind because they required individual attention, especially a cupboard full of my own old preparations. When we returned, we could not get into the laboratories. We were told that all locks had been changed at somebody's order . . . And since at Columbia one left hand never knows what the other does, it was quite fitting that less than six months after they had changed the locks on me at the Medical School, the University gave me an honorary doctorate".

The distinct impression is that the walls of Columbia are covered with *Rhus toxicodendron* instead of the *Parthenocissus quinquefolia* of her seven sister institutions.

On DNA and related matters

Chargaff describes the results of his early analyses:

> "The regularities of the composition of deoxyribonucleic acids—some friendly people later called them the 'Chargaff rules'—are as follows: (a) the sum of the purines (adenine and guanine) [A+G] equals that of the pyrimidines (cytosine and thymine) [C+T]; (b) the molar ratio of adenine to thymine equals 1; (c) the molar ratio of guanine to cytosine equals 1."

He later refers to this paper as

> "my ancient review article on nucleic acids, *announcing* the observation of the complementary relationships in DNA" (emphasis added).

First, if (b) A=T and (c) G=C, it immediately follows that A+G=T+C, so (a) is redundant. Next, let us look at the paper in question, the title of which is *Chemical Specificity of Nucleic Acids and Mechanism of Their Enzymatic Degradation*. The only molar ratios presented in the tables are A/G, T/C, and A/C, never the crucial A/T and G/C. Hidden away in the text is the sentence (emphases added):

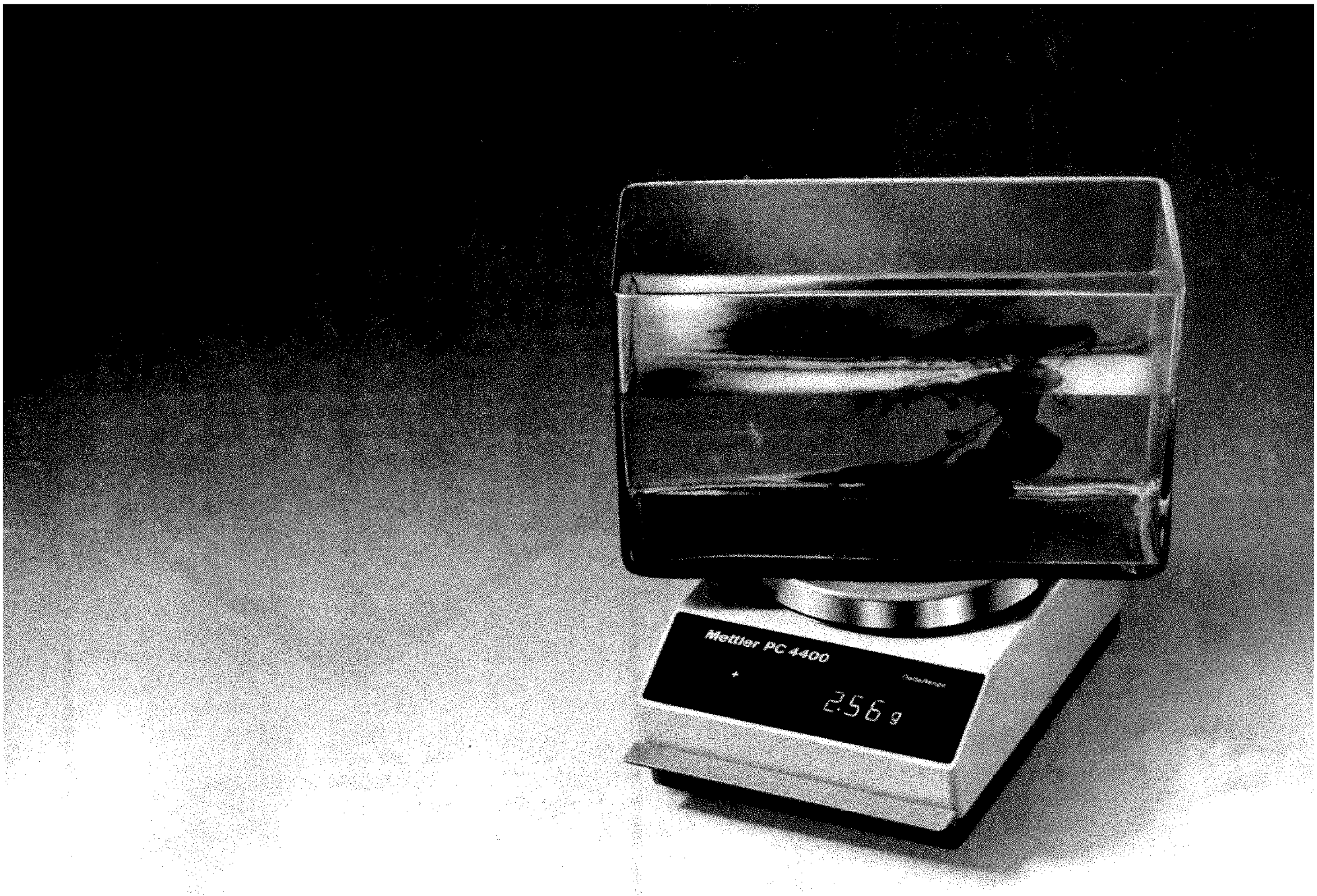
> "It is, however, noteworthy—*whether this is more than accidental, cannot yet be said*—that in all desoxypentose nucleic acids examined thus far the molar ratios of total purines to total pyrimidines, and also of adenine to thymine and of guanine to cytosine were *not far* from 1." [Calculations with data in other tables gives A/T values of 0.90 to 1.20, and G/C values of 0.94 to 1.33].

This hardly seems an *announcement* of complementarity.

This 1950 paper was based on lectures given in 1949. In 1951 there is another paper (*Fedn Proc.* **10**, 654) in which, after noting that A/T and G/C are both "near 1," and (A+G)/(T+C) is "never far from 1," he remarks "whether these are *merely accidental* cannot yet be decided" (emphasis added).

In a 1952 paper, Chargaff *et al.* (*Biophys. biochem. Acta* **9**, 402) give analytical ratios of, among others, G/C of 0.85, 0.90, and 0.93 for DNAs of several microorganisms, results hardly reinforcing the 'rules' with great vigor.

Who, according to Chargaff, made use of his rules? We now move to the



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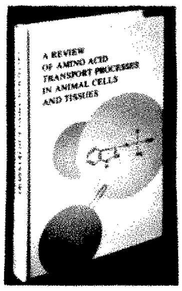

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Cavendish Laboratory of Cambridge University which he visited in May of 1952, where John Kendrew asked him to speak with "two people who were trying to do something with nucleic acids". What happened:

> "The first impression was indeed far from favorable; and it was not improved by the many farcical elements that enlivened the ensuing conversation, if that is the correct description of what was in parts a staccato harangue . . . The impression: one, thirty-five years old; the looks of a fading racing tout, something out of Hogarth ('The Rake's Progress'); Cruikshank, Daumier; an incessant falsetto, with occasional nuggets glittering in the turbid stream of prattle. The other, quite undeveloped at twenty-three, a grin, more sly than sheepish; saying little, nothing of consequence; a gawky young figure, so reminiscent of one of the apprentice cobblers out of Nestroy's *Lumpazivagabundus*. I recognized a variety act, with the two partners at that time showing excellent teamwork, although in later years helical duplicity diminished considerably. The repertory was, however, unexpected. So far as I could make out, they wanted, unencumbered by any knowledge of the chemistry involved, to fit DNA into a helix."

In the case of Crick, this unencumbrance apparently persisted for some time, for as late as 1966 (*J. molec. Biol.* **19**, 548) we find him giving a drawing of a G–A pair featured by a non-bonded H · · · H distance between the bases of less than 1.7Å, a value impossibly small and ridiculously erroneous.

And then:

> "I told them all I knew. If they had heard before about the pairing rules [*sic*], they concealed it. But as they did not seem to know much about anything, I was not unduly surprised . . . I believe that the double-stranded model of DNA came about as a consequence of our conversation."

Now, there are only a very few people who are in a position to know whether or not this belief of his is true. I am one of them. I categorically state that it simply is *not* true. Shortly afterward, when I came on the scene, at such times as they were thinking about DNA, they worried more about the density, the pitch of the helix; pairing, if it came up, was "like with like", not complimentarity. They were not, in fact, even using the correct chemical structures of the bases. When the final model of DNA was discovered—more or less by accident—it wasn't Chargaff's rules that made the model, but the model that made the rules.

Finally:

> "Later, when molecular prestidigitation ran wild, I was often asked by more or less well-meaning people why I had not discovered the celebrated model. My answer has always been that I was too dumb, but that, if Rosalind Franklin and I could have collaborated, we might have come up with something of the sort in one or two years."

Crick's own estimate of how long it would have taken Franklin (by herself) to arrive at a correct structure is:

> "Perhaps three weeks. Three months is likelier. I'd certainly say three months, but of course that's a guess" [see *Rosalind Franklin and DNA*, by Anne Sayre, Norton, New York, 1975, p214].

This crystal balling, whether by Crick or Chargaff, to estimate the time required for the successful solution of a research problem is of course totally fatuous. Chargaff's estimate of two years, however, may safely be expanded to "never", because as late as 1952 he was referring to "enolic hydroxyls" (*Biochim. biophys. Acta* **9**, 399; 402). As everyone now knows, the Watson-Crick breakthrough is based on the fact that someone told them that there are *no* enolic hydroxyls in DNA, and had Chargaff used them an acceptable structure would never have been attained by him.

Some years previous to the "discovery of the century" Chargaff tells us that he "was in love with topology, and all forms of twisted rings filled the office. . ." and that "DNA was pictured as a Möbius strip." He then proceeds to give a wholly erroneous method for constructing such a strip. The love affair was obviously one-sided.

On science and research in our time

> "I would say that most of the great scientists of the past could not have arisen, that, in fact, most sciences could not have been founded, if the present utility-drunk and goal-directed attitude had prevailed".
>
> "Our kind of science has become so dependent on public support that nobody seems to be able to do any research without a handout. If their applications are turned down, even the youngest and most vigorous assistant professors stop all work and spend the rest of their miserable days writing more applications. This continual turning off and on of the financial faucets produces Pavlovian effects and a general neurasthenia that are bound to damage science irreversibly".
>
> "One man or two may decide to study a certain beetle. Whether they do this because the animal is a pest or a biological delight is immaterial. If they find something of scientific interest, there will soon be ten others and more who will do the same. Once there are a hundred men studying the beetle, they will form a society and publish a journal. A society creates a profession, and a profession cannot be permitted to die. It is up to the nation to support it. If the nation can be persuaded, there will soon be a thousand members of the society for the study of the beetle. It is obvious that at this stage the beetle can no longer become extinct, for what would all these experts do who may well outnumber the beetle? Then a foundation will arise whose lay members—influential bankers, society ladies—will neither know nor care whether their function is to help with the eradication or the preservation of the beetles. They know one thing: they must support those who study the beetle. There may even be a Beetle Ball. But what if the beetle disappears after all? The National Infantile Paralysis Foundation has avoided the worst by finally omitting its objective from its name. The American Cancer Foundation may find this more difficult".

Sic Transit

The penultimate section is entitled, *Liber Scriptus Proferetur*, and consists of a dialogue between the author and a "Voiceless Voice". It is a kind of *Höre mit Sinn, was ich dir sage*, and there is much said and much to listen to, mostly depressingly pessimistic, with the twilight glow gradually fading in the dim distance. Perchance the author is indeed a *Greis*.

Even though it may seem that I have some reservations, these, too, fade, with repeated reading of this book. Read it yourself, by all means. It is readable, enjoyable, enlightening, and at times hilarious without being funny. In the spectrum of scientific autobiographies which runs from the unbelievably dull to the delightfully enthralling, Chargaff's shines near the top. ☐

Jerry Donohue is Rhodes–Thompson Professor of Chemistry in the University of Pennsylvania. He spent the academic year 1952–53 as a Guggenheim Fellow at the Cavendish Laboratory, University of Cambridge, UK.

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Thirties science movement

Steven Rose

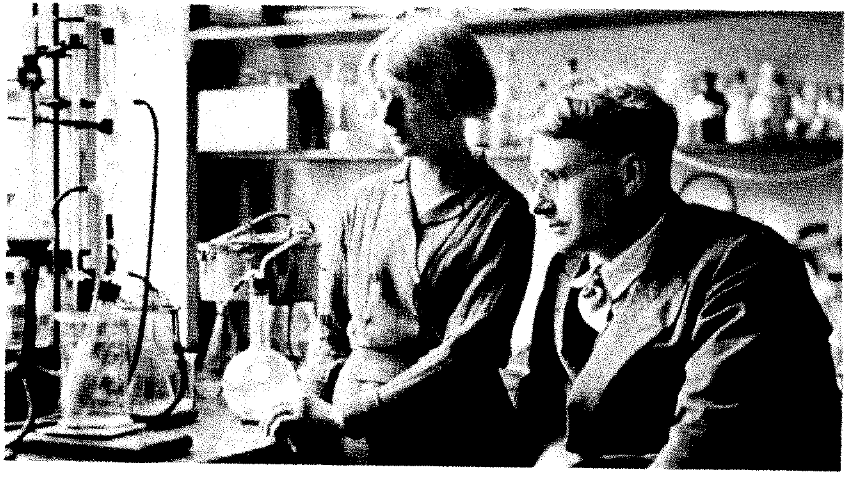
The Visible College: A Collective Biography of British Scientists and Socialists of the 1930s. By Gary Werskey. Pp. 368. (Allen Lane: London, 1978.) (Publication date: 16 November.) £10.

In the history of science as an institution, from the first tentative steps towards the organising of like-minded amateurs in the seventeenth century, to its present-day well-nigh total incorporation into the machinery of the state, the period between the 1914–18 and 1939–45 Wars occupies a position of particular interest and importance. For countries like Britain the Great War demonstrated the need for more decisive state and industrial intervention into the course of science and resulted in the establishment of forms of organisation—such as the Research Councils and Associations—which have remained with us through all the exponential growth phase of science spending which followed, until the present day. This expansion in the scale of scientific effort and rate of scientific advance (limited though it was by the depression of the 1930s), had a series of consequences.

An increase in the number of professional and qualified scientists and engineers and the resultant modification in their work style and class perspectives resulted in the first moves towards scientific unionisation with the National Union (later Association) of Scientific Workers, today merely a nostalgic memory in the maw of Mr Clive Jenkins' Association of Scientific, Technical and Managerial Staffs (ASTMS). In the superstructural world of the intellectuals science could no longer, as it had previously, be regarded with Swiftian or Peackockeian disdain by the literati; what scientists said and thought about the world began to matter: *par excellence* this was the period of Snow's Two Cultures.

By the end of the 1939–45 war and the years that followed, the scientisation of everything had changed the terrain of that battle beyond recognition. As a result of these developments, particularly in the cataclysmic decade of the 1930s, science could no longer remain disengaged from politics at any level. What science was financed, and how, was a political question; what science discovered, and how it proceeded, were political and ideological questions; what scientists said—about science and about politics—began to matter in a way that had never been the case before.

These changes excited, engaged, divided the 'scientific community'—by which one normally means that visible part of the profession which is university-based and regards it as its role to provide intellectual leadership. The divisions transcended academic debates and spilled out even from the pages of *Nature* into popular writings and political organisations. On the one hand there was a combination of mystically minded idealists like Jeans and Eddington, traditional conservatives with a dislike for the radical changes that were beginning to show in the scientific profession, like A. V. Hill, and proselytysers for a romantically conceived 'freedom' in the 'republic of science' like Baker and Polanyi. On the other, a formidable group of intellectuals with a range of talents within their disciplines and political and organisational skills that were to play their part in the total transformation of science in the decade that followed—Bernal, Haldane, Hogben, Levy, the Needhams, the Fremlins, the Piries, the Woosters, aided by a new species of science writers with access to the daily press like Crowther and Ritchie Calder.



Dorothy & Joseph Needham in 1927 at the Dunn Biochemistry Institute, Cambridge

The history of the science movement of this period is central to an understanding not merely of the political and cultural ferment of the 1930s, but to the appreciation of the intellectual and organisational origins both of British state science policy as it developed in the post-1945 period and of the new radical science movement which emerged in the 1960s. It is both surprising and slightly embarrassing that, although studies of the literary intellectuals of the 1930s have been so common, we should have needed to wait so long for the history which Gary Werskey has attempted in *The Visible College*.

Werskey's book has grown out of the thesis he embarked upon more than a decade ago now. His method is to analyse what we would today call the radical science movement, then referred to as the 'social relations' or 'social functions' of science movement, through a discussion of the activities and careers of five key figures; Bernal, Haldane, Hogben, Levy and Joseph Needham. He briefly traces the biography of each before the 1930s, trying to identify those factors in their development which led them eventually to socialism and for several, membership of the Communist Party. For Werskey's analysis the intellectual pivot around which the book revolves was the now famous International Congress of the History of Science in London in 1931, to which the Russians sent a highpowered delegation headed by the major theoretician and Old Bolshevik Bukharin, which transformed the younger British scientists' understanding of the social processes of science by presenting them with a worked-out analysis of the historical and economic determinants of Newton's *Principia* as rooted in the needs of the emergent English bourgeoisie of the seventeenth century. The externalist analysis of the history of science was born.

In two central chapters Werskey discusses first the theoretical issues for the left scientists—essentially disparate attempts to define the relationships of science to dialectical materialism (particularly Haldane) and the social functions of science (Bernal)—and second, their practical work in the Association of Scientific Workers and, later, the Cambridge Scientists' Anti-War Movement. There is a brief account of the War and post-War years—disappointingly thin compared with the wealth of published autobiographical material available here—and a concluding chapter significantly entitled "Coming in from the Cold". Finally, an afterword very briefly summarises the heritage of the movement in the Janus-form of the almost total acceptance of state planning for science in

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a capitalist Britain, and the rebirth of the radical science movement in the late 1960s around the issues of chemical and biological warfare and later the founding of the British Society for Social Responsibility in Science. Here, where my generation's autobiographies begin to interlock with those of the 1930s movement, Werskey leaves his story.

One should not underestimate the importance, or the difficulty, of the task Werskey sets himself. But I believe that in the last analysis he fails, and that the reasons for this failure are not uninstructive.

Firstly, the book is very uneven. It changes style and method as it progresses—the inevitable consequence perhaps of an overlong gestation period, and one during which the author's own political and intellectual positions *vis-à-vis* his protagonists and their goals have themselves undergone a considerable evolution—as he himself would, I am sure, be the first to agree. Thus, we move from a sort of psychologising biographical enquiry, seeking to root the socialism of Bernal and his colleagues in their early childhood, to an intellectual debate with the theoretical postulates of dialectical materialism as they were interpreted during the 1930s, to an account of a social movement and a puzzled attempt to come to terms with why left intellectuals in the 1930s saw the Communist Party as the proper and inevitable place to be.

Hyman Levy broadcasting a science programme for the BBC in the 1930s

Haldane attempting to stir up a 'United Front' meeting in Trafalgar Square in 1937

The book is at its strongest when it discusses social movements like the Cambridge Scientists' Anti-War Movement. Elsewhere, it falters. It would be wrong to argue that only an insider could have written the history that Werskey has attempted, although it is true that a certain unease about the young American historian of science amongst the potential subjects of his enquiry has limited his access to some of the most interesting documentary material of the period, but nonetheless a consistent method of approach and a more basic sympathy with the intellectual as well as the social tasks that the Social Functions of Science movement attempted would have avoided some of Werskey's problems. If he had based his analysis on the changing objective status of British science *vis-à-vis* British society he would not have been forced back into the sort of psychologising that wants to seek common features in the marginality of his key figures (to argue that Haldane's Scottish connections made one who was so solidly born into the British scientific and political aristocracy and Oxford society 'marginal' is surely stretching a point). His account of the Cambridge social scene of the 1930s is too uncomfortably reminiscent of Jim Watson's 1950s version in *The Double Helix* to carry total conviction. His essential unconcern for the scientific problems which Haldane, Bernal, Hogben, Levy and Needham set themselves also leaves him unable to see the importance that they felt holistic methods opened up within their own subject areas, by contrast with either mechanical reductionism on the one hand or idealism of the Mach/Jeans/Eddington type on the other. Yet much of their best theoretical writing, and that of their colleagues and followers was addressed to just these questions. The analysis of the social functions of science was merely one aspect of the challenge that the left scientists presented; the issues and approaches that they opened in theoretical, population, developmental and human biology remain a profound and to date inadequately discussed theme (partly, it is true, due to the tendency of Haldane to mask subtle truths in outrageous *obiter dicta* and of Bernal to subordinate many of his theoretical concerns to those of the intellectual defence of the Soviet Union even where it was indefensible); nonetheless Werskey largely ignores this aspect of the group's work.

A further problem to Werskey is the role of the Communist Party and the relationship of his subjects to it. Again this problem partly derives from the technique of the tracing of five interlinked careers rather than the social movement and its objective base; he thus has to look for individual motivations rather than changing social and economic circumstances. But also, one feels that Werskey has not been able to shake himself entirely free from a cultural and political background in which the American cold-war assumptions of his early training was followed by the essentially spontaneist radicalisation of the student movement of the 1960s. For him therefore, the Communist Party always appears as an external force motivated by Moscow, involved in Machiavellian schemes to control, neutralise or otherwise manipulate the misguided intellectuals. This is apparent in his choice of language; he speaks of Bernal and his "cohorts", a "left wing coterie" and so forth. Nor is his overall grasp of international communist theory and politics in the 1930s and 1940s absolutely sure, even on issues impinging directly on the science movement—for instance one should not argue that Stalin made a "sudden decision" in July 1948 to put T. D. Lysenko in charge of Soviet biology, especially after the re-analysis of the Lysenko affair in recent years by Lewontin and Levins, and by Lecourt.

What is of real interest is the essentially progressivist image of science that the old left had: science was inevitably in contradiction with capitalism and on the side of socialism. This was a view shared—and indeed Werskey hints at this—by the Communist Party with its belief in the 'scientific' nature of its version of Marxist theory. The alliance between socialist scientists and the Communist Party was thus inevitable. Indeed, it was a great deal more durable than that between non-scientific intellectuals in the 1930s and radical or Communist politics. The interesting point is the importance both sides attached to this alliance—for instance, natural scientists formed a significant segment of the Editorial Boards of the main theoretical journals of the Party and non-Party left in the 1930s and 1940s.

One of the most important differences between them and us is that the organised left no longer lays claims to such people; today its theoreticians are sociologists rather than scientists. Few of the leading cadres of the new radical science movement in Britain are active in scientific research in the same way as was the generation of the 1930s. The reasons for this change are surely connected with a completion of those tendencies in the development of the social functions of science which the 1930s heralded.

While Werskey's book remains the only history of the 1930s science movement, this analysis will, however, remain incomplete. ☐

Steven Rose is Professor of Biology at the Open University, Milton Keynes, UK.

Shades of Audubon

C. M. Perrins

Audubon: A Biography. By John Chancellor. Pp. 224. (Weidenfeld and Nicolson: London, 1978.) £6.95.

LAST year, an original set of Audubon's plates *The Birds of America* was sold at auction for a little over £200,000, the highest price ever paid for a copy of a printed colour-plate book. These extended-coffee-table works have captured the imagination of the world and a number of biographies have already been published. One therefore picks up another biography of Audubon with a little caution. The work under review, however, is a useful history of this man. It traces his family history and his early life in detail, following him through the period when he struggled to make a name for himself to his later life when he was already "a legend in his time."

Audubon was born in 1785, an illegitimate son of a French sea-going father. His mother is of uncertain history; she was called a "creole", but at that time this could have meant a number of things, from half-caste to a person whose family had lived in Latin America for several generations. The family background was one of the slave-owning communities of the southern states and Caribbean; indeed Audubon's father was for a time a slave trader himself. The father and two children returned to France at the time of the French Revolution and the young Audubon was brought up, largely by a stepmother, during these difficult times. He did not return to the New World until the age of 28. By this time he was already a dedicated naturalist and was drawing birds. Once in the States, he devoted his time to hunting, shooting and drawing to the extent that his business ventures failed with dismal reliability. He tried to run a number of merchant stores, moving westwards to Kentucky.

Always he hunted, painted and drew. In the early years he did not do so with any clear plan in mind for publication. However, such a thought may have been sown at a meeting with Alexander Wilson in 1810. Wilson, the first man to publish a book of coloured illustrations of American birds, approached Audubon to seek a subscription for his work (to be published as a serial). Nevertheless, another decade and a half were to pass before Audubon tried seriously to follow suit. During this time his business ventures continued to fail and he and his family were often destitute. Although Audubon sometimes made ends meet by drawing and painting portraits, he never made much money from his natural history work. His wife, Lucy, whose marriage settlement he had long since spent, was often the mainstay of the family, earning a living as a teacher while her husband disappeared into the bush on hunting trips.

Things changed at last in 1826 when Audubon arrived in Liverpool and started to display his paintings in earnest. He met many enthusiastic subscribers for his proposed work and, after finding a publisher who was capable of doing the difficult work of preparing the plates (an Edinburgh firm failed in the early stages and most of the work was engraved by the London firm of Havell) things never really looked back. True, from time to time the number of subscribers dropped (at one time 50 of the 180 subscribers failed to renew their support) because Audubon was wandering in search of further birds and not trying to maintain or increase the number of subscribers; but he was never really short of funds again. By 1831, at the age of 46, Audubon returned to the United States a celebrity. In all *The Birds of America* comprised 87 parts, each of 5 plates—435 plates figuring 489 species. The first part appeared in 1827, the final one in 1838.

Audubon continued to visit England where he had a son overseeing the production of his work at Havell's. He continued to undertake expeditions searching for more species and he started new projects, including the production of an octavo edition of his *The Birds of America* (1840–44), his journals (*Ornithological Biography*, 1831–39), and *The Viviparous Quadrupeds of North America* (1845–48). Many of these were undertaken with assistance of others. The text for his main work and for his journals was heavily re-written by the Scotsman William MacGillivray, himself author of an important work *A History of British Birds*; the text for *Quadrupeds* was written by the Rev. John Bachman, a long-time friend. Audubon died in 1851 before the completion of a new version of this work.

Barn Owls

Plate CLXXI of *Birds of America*

Painting of Audubon by his son

All this has been said a number of times in the numerous biographies. However, the work reviewed here does perhaps stand apart from some of the previous works which have tended to idolise Audubon. John Chancellor is more inclined to be openly critical of parts of Audubon's life. The artist made many enemies during his life and not without cause. He must at times have been an exceedingly difficult person to put up with. He was indolent and unfaithful to his trading partners. He treated his family badly. Even when he was becoming established as an artist he had little loyalty to many of his aides. The backgrounds for his artwork were usually done by other artists; they seldom got much credit and sometimes their names were deleted from the plates. His journals are contradictory and, to put it as politely as possible, Audubon tended to over-embellish the happenings on his journeys. He tried to put himself across as a colourful woodsman. Although it is not really fair to criticise Audubon for lack of conservation interests, as the idea of conservation barely existed at that time, he was wildly trigger-happy. A place where he shot fewer than 100 birds in the course of a day was rated "very poor for birds". It is a little wry

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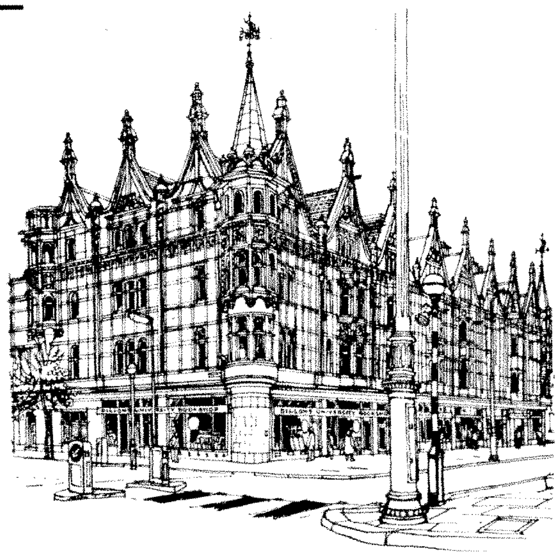

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that a man who shot anything and everything should have given his name to the greatest conservation organisation of our time. On the credit side, his field observations were accurate compared with other contemporary works.

I would have preferred the author to say rather more about the artwork itself. As with the other aspects of Audubon's life, it has both admirers and detractors. Again, it is necessary to set the artwork in its time. There was very little else and what there was was stilted and un-natural, a point that Audubon was quick to stress. Not that, to the reviewer, many of Audubon's own paintings are of great merit in this respect. Many of the birds are depicted in grotesque attitudes which they could never achieve in life; this is especially true of the larger birds whose necks he bent into odd shapes to get them on the page. Perhaps the single most effective aspect of the artist's work was to draw the birds on so grand a scale, the enormous elephant-folio size contributes to the impact of the plates.

The work reviewed here is profusely illustrated. Many of the illustrations are not drawings of Audubon's but works by other artists depicting, for example, places which he visited. Sadly many, presumably for reasons of cost, are monochrome not colour. In fact there are only nine colour reproductions of Audubon's bird work and two of his mammals. The reviewer would have welcomed slightly more detailed documentation and a larger bibliography. Nonetheless, the book makes interesting reading and will be appreciated by many who admire the artist's work and wish to learn more about him. The author has produced a thoroughly readable and attractively produced book. ☐

C. M. Perrins is Director of the Edward Grey Institute of Field Ornithology, University of Oxford, UK.

Anecdotal history of Kew Gardens

William T. Stearn

In for a Penny. A Prospect of Kew Gardens: Their Flora, Fauna and Falballas. By Wilfrid Blunt. Pp. 218. (Hamish Hamilton and Tryon Gallery: London, 1978). £7.95.

WILFRID BLUNT is a master of anecdotal history and biography, as his works on such diverse characters as Sidney Cockerell, King Ludwig of Bavaria, Carl Linnaeus, Felix Mendelssohn, John Christie and G. F. Watts testify. In the Royal Botanic Gardens, Kew, he has found yet another subject for his witty light-hearted versatile scholarship and an approach which he correctly and disarmingly describes as "personal, capricious, irreverent" and "perhaps even prejudiced, for I have always had a kind of love–hate relationship with the botanists". His book is an entertaining combination of guide-book and history, well written and profusely illustrated.

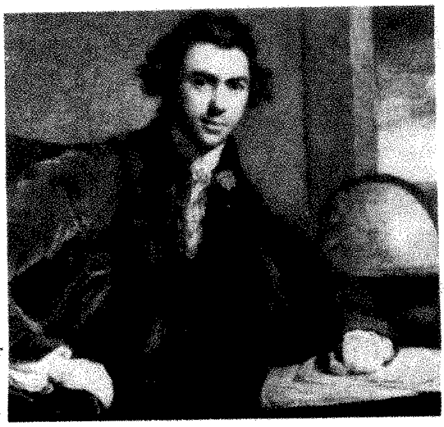
As a botanic garden, Kew, although the mother of gardens in many parts of the world, seems young by comparison with the botanic gardens of Oxford (founded in 1621) and Chelsea (founded in 1673) and even more so with those of Pisa (founded in 1543) and Padua (founded in 1545). All these had originally a medicinal and teaching intent. Kew, which goes back as a botanic garden no further than 1760, owed its existence less altruistically to royal pleasure, beginning with Queen Caroline, wife of George II, whose pet poet and tame hermit Stephen Duck (1705–56) has provided Blunt with material for an amusing marginally relevant chapter.

The horticultural zeal of Frederick, Prince of Wales, and his Princess, Augusta, determined the future of their royal Kew estate. Without them it could never have become the world's finest botanic gardens. In 1959 the Royal Botanic Gardens celebrated the engagement of a Scottish head gardener, William Aiton, as the founding of Augusta's Kew botanic garden, although Sir William Chambers, her architect, writing in 1763, had stated that the "Physic or Exotic Garden was not begun before the year 1760". Between 1757 and 1762 Chambers embellished the Kew park with a diversity of structures, of which the orangery and the pagoda stand as his most conspicuous monuments. Augusta's botanic garden occupied some nine acres of the present 300 acres forming the Royal Botanic Gardens. Blunt's first five chapters deal with this Hanoverian period and he joyfully incorporates its scandalous rumours about an affair between Augusta and her horticultural and botanical adviser, Lord Bute.

Augusta died in 1772. Under her son, George III, Sir Joseph Banks (1748–1820) took control of Kew, sent collectors far afield to enrich it with new plants and employed his botanist-librarians, Solander, Dryander and Robert Brown, to classify and name them and an artist of genius, Franz Bauer, to paint them. The result of their endeavours was the *Hortus Kewensis* published as the work of W. Aiton and his son W. T. Aiton, "the names of native dunces being suffered to usurp the place belonging to those of the genius and talent of another land", according to Ker-Gawler in 1823. (The specimens on which this monumental work was based are in the British Museum (Natural History), not Kew.)

Banks gave the Kew garden an international repute. Unfortunately, as Blunt says, "after the death of Banks in 1820 Kew entered upon the two darkest decades of its history. Indeed it sank so low that at one time what Augusta and Bute had so lovingly created, what Banks had so skilfully nurtured, seemed about to decline into kitchen gardens to provide cabbages for Kings" and might have ended as a built-up area.

The crisis came in 1838. Few of the many visitors to the garden and few of the research workers in the herbarium, libraries and laboratory know that, but for the efforts of a few influential and far-sighted men in the years 1838–42, they would have no Royal Botanic Gardens, Kew, today. The gardens were then under the control of the Department of the Land Steward, who was the Earl of Surrey, and the government, which would have been glad to be rid of them, set up a committee of enquiry for the purpose. It included the Earl of Surrey's head gardener but he was an ineffectual counterweight to Professor John Lindley, a man not only of great botanical knowledge and horticultural experience but also of forceful character, forthright speech and a vigorous lucid style, and Joseph Paxton, the Duke of Devonshire's head gardener, likewise a man of independent views. Lindley's report, advocating the conversion of the gardens into a public institution for promoting botanical science, was not what the government and young Queen Victoria wanted. Public indignation at the proposed destruction, however, led to the adoption of Lindley's views on the function and future of Kew, and in



Sir Joseph Banks

Joshua Reynolds, about 1773

Sir William Hooker

Linnaen Society, London

1841 William Jackson Hooker came from Glasgow to be the director of the Royal Botanic Gardens, which under his guidance then developed into the botanical centre of the expanding British empire, as Lindley had hoped. I think Blunt does not give Lindley enough credit for his major contribution to the saving and development of Kew.

That development makes a varied story. Blunt tells it in fragments as he conducts his readers on a tour of the Marianne North Gallery, the Cambridge Cottage, the T-Range, the Herbarium and so on, though failing to do more than view from a distance the Jodrell Laboratory, which is a pity, as its forensic associations alone would have given him interesting material. Moreover, here C. F. Cross and E. T. Bevan did the early work leading to their discovery of viscose in 1892 and so to the rayon manufacturing industry. The celebrated plant anatomist and palaeobotanist, D. H. Scott, Honorary Keeper of the Jodrell Laboratory from 1892 to 1906, is likewise nowhere mentioned. Thus, although Blunt provides an excellent general view of Kew as a visitor sees it, together with a good historical background, the scientific work done there is largely and deliberately outside his chosen field. Indeed he describes his book as "an aperitif to the feast that Kew provides, not a meal to be consumed in the gardens."

Blunt asks "my friends the botanists to forgive a little gentle badinage at their expense". They are, he says, "kind and delightful people"; he should therefore have asked more of their help over his shaky acquaintance with plant names. Thus, he attributes the coinage of the old Greek herbgatherer's name ὀρχισ (testicles) to Theophrastus, who simply used the common vernacular names of his times. "Why", he asks, "did they [the botanists] make Orchis *feminine* when the Greek word, and what it stands for, are masculine?". In fact modern botanists merely follow classical usage. The Ancients when using the word *orchis* not literally for humans but metaphorically for plants made it feminine in gender because most of their plant names were feminine and an association with a feminine noun such as ἐλαια (olive), *olea* or *herba* was implied. Thus Pliny wrote "mirabilis est orchis herba sive serapias, gemina radice testiculi simili." As regards the names of multigeneric orchid hybrid groups (see p177), the proposal to name these by attaching the termination *-ara* to the surname of a person was devised by E. A. Bowles about 1909, and put forward at the Brussels Botanical Congress of 1910, by which time such unwieldy condensed formulae as *X Brassocatlaelia* and *X Sophrolaelio cattleya* already existed. However, as Blunt says, "all of this has little or nothing to do with Kew".

It is unfortunate that he should repeat the old libel that work in the Kew Herbarium "adds to the misery of gardeners by constantly changing the scientific names of familiar plants". There is still room for a book covering the multifarious activities of Kew, which have included efforts to preserve unchanged the scientific names of plants. On p114 Kew and South Kensington are confused in a quotation from Beatrix Potter's diary; the entomologists Kirby and Waterhouse and the anatomist Sir William Flower were never members of "Kew's behind-the-scenes staff"; they worked in the British Museum (Natural History).

The illustrations delightfully supplement the text; one portrays the director Sir William Thiselton-Dyer, "whose sense of humour was limited though that of his self-importance was not", as Blunt observes, parading in a constable's uniform with his garden constables. William Purdom, the rebellious Westmorland gardener who defied the autocratic Sir William, subsequently became a plant collector in China for Messrs Veitch and the Arnold Arboretum and travelled with Reginald Farrer. Indeed the history of Kew teems with names illustrious in botany and gardening, as well as some notorious, and Blunt has gathered anecdotes about them from multitudinous sources to produce a lively "personal, capricious, irreverent" book. ☐

W. T. Stearn is a Visiting Professor in the Department of Botany, University of Reading. His continuous unofficial but close association with the Royal Botanic Gardens, Kew, began in 1930.

Crusade against malaria

L. J. Bruce-Chwatt

Mosquitos, Malaria and Man: A History of the Hostilities since 1880. By Gordon Harrison. Pp. 314. (John Murray: London, 1978.) £8.50.

THIS study by a "military and political historian and an environmentalist" (as stated in the author's preface) covers the period 1880–1976 of man's fight against malaria, the world's most prevalent tropical disease.

The book starts with the description of the discovery of the malaria parasite in Algeria by the French military surgeon Alphonse Laveran; it provides interesting and little known information on the background to this event and points out the difficulties that Laveran had in convincing the medical pundits (including William Osler) of the importance of his finding.

The next few chapters are devoted to Ronald Ross, undoubtedly the most colourful personality in the history of tropical medicine. Ross's "pilgrim's progress" from his first contact with Patrick Manson, through his fumbling, laborious and then inspired work in India, to the crowning glory of the Nobel Prize awarded for the discovery of the transmission of malaria by mosquitos, is told with much understanding and fluency.

Ross's bitter quarrel with the Italian investigators leads the author to an excellent appraisal of the major scientific contributions of Golgi, Grassi (Ross's "bête noire"), Dionisi, Bignami and Bastianelli. Here once again many interesting details, based on thorough research of original sources enliven the narrative. Robert Koch's little known and less than illustrious role at this stage of the history of malaria is discretely but skilfully reported. There follows the chapter on "three lives of the Plasmodium" which takes the reader through the explanation of the cycle of transmission of the human malaria parasites, including the cryptic tissue phase revealed in 1948 by Shortt and Garnham.

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Ronald Ross

Photo: London School of Hygiene & Tropical Medicine

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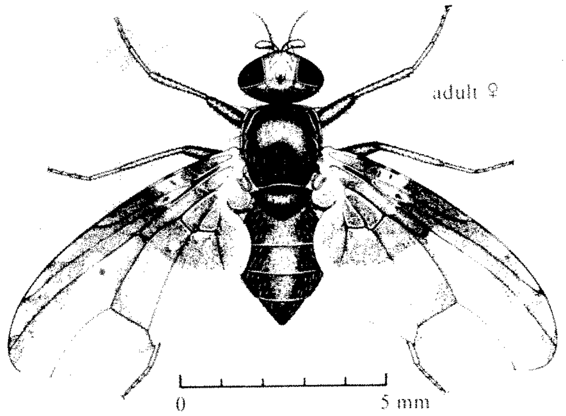

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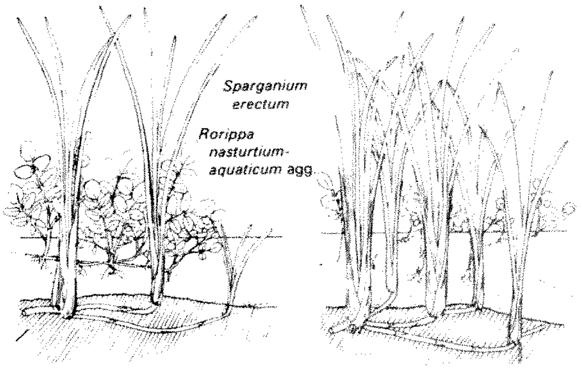

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mosquito breeding places in Sierra Leone, through Sir William Macregor's enlightened sanitation measures in Lagos, Malcolm Watson's brilliant successes in Malaya and then the controversial malaria control project of Indian Army's barracks at Mian Mir, near Lahore. (The discussion of the latter episode is particularly well researched and lucid).

A good popular account of the natural history of mosquitos precedes the description of the successful control of yellow fever and malaria in Panama, by General William Gorgas. This is also well told and provides many little known details of Gorgas's splendid effort in spite of the differences he had with Goethals, the commander of the Panama zone.

The next important phase of the fight against malaria opened in the early 1930s in Italy and the conflict between Angelo Celli's ideas imbued by his social reformer's spirit and Alberto Missiroli's growing faith in the new technology of control by elimination of adult Anopheles from human dwellings, is well brought out. The powerful stimulus of L. W. Hackett of the Rockefeller Foundation's research team in Italy is given a proper place in this part of the story.

Further chapters present the dramatic impact of malaria epidemics in Mauritius, Sri Lanka (Ceylon), Brasil and Egypt in the 1940s and the dawn of the technique of insecticidal spraying of houses as a promising method of control. The apparent failure of the Sardinian project of "species eradication", carried out between 1946 and 1951, was a prelude to the emerging concept of malaria eradication programmes which were set up at that time in Greece, Venezuela and elsewhere.

The last two chapters, entitled "India the model for the world" and "Relapse", cover the past 20 years of the global malaria eradication programme launched, coordinated and supported by the World Health Organisation since 1957. Here the author attempts to deal in a simplistic manner with all the complex problems that bedevilled this great and overambitious international endeavour. This part of the book fails to appreciate the many positive achievements of malaria eradication and seems to concentrate on pointing out the various deficiencies and errors—some true, others imaginary. However true certain remarks may be, the author's tendentious debunking of this major effort, with the air of someone who knows all the answers, is grossly unfair. His critical comments on the recent realistic appraisal by the WHO of the need to assess the costs of malaria control in relation to the means of the tropical countries concerned, reveal his scant acquaintance with the socio-economic aspects of health problems of developing countries.

Oddly enough, there is no mention of the fact that the most stringent criticisms of the strategy of the global programme of malaria eradication came from the remarkable, self-auditing study of the World Health Organisation, namely the well known Annex 13 of the Director's General Report to the Twenty-Second World Health Assembly in 1969.

The achievements of the past 50 years of chemotherapy of malaria as an important weapon for prevention and treatment of this disease are omitted from this otherwise comprehensive study.

In summary, this is a patiently researched and very readable story of events that formed the milestones of the relatively recent knowledge of the transmission of the agent of malaria and its control by the attack on the vector. It is regrettable that the appraisal of all aspects of the attempted global malaria eradication is somewhat unbalanced, by concentrating on its failures.

There are several minor errors in the names of persons or titles of books (for example, Beauperthuy not Beauperthy; Daniels and not Daniel; Macdonald not Mac Donald; Ross's romance was *The Spirit of the Storm* not *The Spirit in the Storm*). Some footnotes are perplexing: thus, the one on p40 says: "Ross knew that it was the female that sucked blood but military man, that he was, he persisted in calling her 'he' ". A few instances of purple prose such as Ross's "adrenaline pump" or his "ecstasy of Ego" are jarring in the generally clear and pleasing style of a book that should nevertheless interest a wide circle of readers. The illustrations are well chosen: the copious notes are generally relevant and the list of references is valuable even though it shows some inexplicable lacunae.

The blurb on the cover mentions that "The extraordinary story of the crusade against malaria is told here for the first time". This is simply not so, because several excellent books on the subject were published during the not too distant past: Paul Russell's *Man's Mastery of Malaria*, of 1955, is still one of the best of them. Moreover the recent ups and downs of malaria eradication have been dealt with fully in published reports of the WHO and in numerous other articles. ☐

L. J. Bruce-Chwatt is Professor Emeritus of Tropical Hygiene in the University of London, a former staff member of the WHO, and a member of the WHO Expert Panel on Malaria.

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Ecological sanity

Kenneth Mellanby

Reconciling Man with the Environment. By Eric Ashby. Pp.104 (Oxford University Press: Oxford and London; Stanford University Press: Stanford, California, 1978.) £4.25; $7.95

LORD ASHBY is a distinguished botanical research worker. He was Professor of Botany in Sydney and in Manchester. He was active in establishing the Society for Experimental Biology in the 1930s. He was also the first chairman of the Royal Commission on Environmental Pollution, set up by the British government in 1970 to "advise on matters, both national and international, concerning pollution of the environment; on the adequacy of research in this field; and the future possibilities of danger to the environment." The experience gained in this career has given him an ability to survey the environmental scene with unusual perspicacity.

In this book Lord Ashby is particularly concerned with the ways in which public opinion may stimulate governments to cope with hazards to the environment. He describes this as a "chain reaction", in which the interest of the public is first ignited by the media, then the hazards are assessed by scientists and economists, and, finally, political action is taken. This action is often based on a compromise between opposing interests, and may take into account both objective data and subjective values. In general the British pragmatic approach, with attention to concrete effects on man's surroundings rather than on rigid standards setting levels of emission, is shown to be most effective.

Successes and failures to protect the environment are illustrated by several examples. Lord Ashby describes graphically the struggle over Upper Teesdale in the north of England, where an area of unique botanical importance was the most convenient—and least expensive—site for a reservoir, needed for the industrial development of an area of potentially high unemployment. Although the reservoir was eventually built, the botanists and other conservationists who opposed its construction were given a good hearing and substantial modifications were made in the scheme to try to reduce its ecological impact. Funds for research in the area were also provided by the commercial interests most involved. Lord Ashby infers that this demonstrates how, by the 1960s in Britain, public opinion had been educated. It is suggested that at an earlier date industrial necessity would have been encouraged to ride roughshod over the site, destroying the Teesdale Sandwort and the Spring Gentian.

Of more general topics, the examples of the great improvement of the quality of the air in British cities, and the moderate improvement of the state of the water in British rivers, both in the past quarter of a century, are quoted. These improvements have cost both industry and the public a considerable amount of money, something which might have not been tolerated fifty or a hundred years ago. In the nineteenth century many attempts to persuade Parliament to legislate were unsuccessful before the passing of the first Alkali Act (controlling little else but acid emission to the air) in 1867. The more drastic Clean Air Act of 1956 was generally welcomed, even if some of its most stringent provisions have not yet been adopted in many parts of the country.

In recent years there has been serious controversy about waste disposal, particularly of poisonous substances. Lord Ashby's Royal Commission realised this was a danger, and so reported strongly but soberly in its 1971 report. The government did nothing—the programme for parliamentary legislation was full up. The matter was then taken up, more shrilly, by various voluntary bodies of citizens concerned with the environment. They made the case more emotionally, but still relied for the main on a reasonable assessment of the dangers. Nothing happened—Parliament was still too busy. Then a large number of drums of cyanide were were found, with their labels inefficiently removed, in an area of wasteland used as a childrens' playground. It was alleged that this "flytipping" occurred with the tacit encouragement of the driver's employing company. There was an immediate outcry, and within days Parliament passed a bill to prevent a recurrence.

However, I think that Lord Ashby may be overstating the contribution to the protection of the environment by the media, and of any widespread change in "public opnion". In the case of the toxic wastes, the drum of cyanide were a real and easily demonstrable danger, which dramatically highlighted the Royal Commission's warnings. Often it seems that there is only a public outcry about environmental problems after they have been solved by backroom experts using unglamorous means. This may be illustrated by changes in air pollution. It is generally believed that the improvement in Britain occurred because of action following public outcry, when it was reported that the great London "smog" of 1952 killed off, prematurely, some 4,000 elderly, ailing and bronchitic citizens. The resulting Clean Air Act is thought to have been the main instrument of change. In fact London's air even by 1952 was far cleaner (or, perhaps, less filthy) than it had been in the nineteenth century, in the days of Dickens. The improvement was mainly because it became cheaper and easier for industry to use clean fuels like oil, gas and electricity, and because the competent and well informed Alkali Inspectorate was beavering away controlling industrial emissions years before the Act was passed in 1956. At the same time many householders had already stopped burning raw coal before regulations compelled them to do so. Legislation was useful, but only in accelerating a change already taking place.

The situation regarding pesticides is also interesting. Lord Ashby says that had not Rachel Carson published *Silent Spring* in 1962 "public apathy about the hazards of pesticides might have persisted a decade longer". This is quite true, but is hardly relevant to the way in which pesticides were controlled, at least in Britain. Here naturalists and ornithologists in particular had been concerned with the deaths of thousands of seed-eating birds each spring from about 1955 onwards. The situation had been investigated by them and by scientists in government laboratories, and seed dressings of aldrin and dieldrin on spring-sown wheat implicated. Some time before *Silent Spring* was published the voluntary ban on these seed dressings on spring wheat was operated, with the result that the bird deaths were greatly reduced. Even before that the successful Pesticides Safety Precautions Scheme was set up with the result that no new chemical causing comparable ecological effects on non-pest species has been introduced. It is possible to argue that the wide public reaction to pesticides, sparked off by Rachel Carson, has generally been counter-productive. Before the book appeared, informed public opinion had taken effective action. After the book aroused uninformed opinion, many, particularly in developing countries, have been denied the use of safe pesticides and so the incidence of, and death toll from, diseases like malaria has greatly increased.

However, I only disagree with Lord Ashby in the way we define "public opinion". Effective environmental control has required the support of the informed public, but not of the masses

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in general. I realise that this view will today be castigated as 'elitist' but it is nevertheless true. Where mass opinion has been inflamed, overreaction has often occurred, unsubstantiated statements have been put forward, and eventually there has been a 'backlash'. This is being experienced in many Western countries today, where the majority of the population are becoming bored with the environment. The uninformed and over-reacting 'environmentalists', preaching doom and gloom, are becoming increasingly discredited.

Lord Ashby is certainly too well informed to have any truck with the doom and gloom brigade. He considers that man has been, to some extent, reconciled to his environment, and that this is a "qualified success story". He does not conceal the fact that real damage occurs, and he indicates how this damage may be mitigated But he concludes his book by pointing out other, real reasons for concern. These are man's prodigal use of non-renewable resources and the difficulty those people with different ideologies have of living together in harmony. The final, and greatest danger, is thus political. The only hope is that mankind will learn from the qualified success in the environmental field. Here the cumulative effects of wise decisions (what Lord Ashby calls "wise medium-term microdecisions, each clarifying the shape of the decision that needs to follow") has produced a promising result. We can only hope that the problems of diminishing resources and political incompatibility will be solved in the same unspectacular way, by the efforts of the responsible minority, not be a tumult promoted by the media. □

Kenneth Mellanby is editor of the International Journal Environmental Pollution.

Politics of atomic energy

L. Rotherham

Nuclear Power and the Energy Crisis: Politics of the Atomic Industry. By Duncan Burn. Pp. 352 (Macmillan: London, 1978.). £12.

In his preface, Duncan Burn suggests that reviewers will be able to point out that some things are left out from this book but that the omissions will not affect the argument presented. There is a selected bibliography which includes about 100 items, and references in the text must number 700–800 Although the same reference may appear several times, no one would wish to say that the author has not carried out his work thoroughly. Some references unfortunately are useless: for example, taking only chapter 10, we find ref. 188/40—"I am following in this notes of discussions I had . . . " or 188/53—"This view was expressed to me early in 1964 by a member of the CEGB"—why not say which member, so that it can be authenticated?; or 191/109—"I had discussions at the time".

One would not wish to question the authenticity of the author's statements but he might have heard what he wanted to hear and we cannot say. Where he refers to published work, his excerpts seem to be exactly correct but his comments and his selection seem to be aimed to sustain his own opinions, and in the light of history that hardly seems necessary.

If this justification is the first theme of the book, a second is that the assessment of tenders for the Dungeness B power station was mishandled by the Central Electricity Generating Board (CEGB) and the Atomic Energy Authority (AEA), mainly due to the overwhelming influence of the AEA; and finally, it is suggested that the Government shares in the blame by interference in technical decisions where it lacks competence. These three themes are linked, of course.

The tenders for Dungeness B were received in 1965 and at the time virtually all British experience was in gas-cooled nuclear reactors, whereas in the United States nearly all experience was in water cooled reactors (LWRs) and in both cases the early experience was taken from military applications. In Britain, Calder Hall (a gas-cooled station) had been operating well since 1957 and rather larger stations had been commissioned for the CEGB in 1962 and 1963 These stations are still operating. Capital costs of the CEGB stations were higher than had been assumed but, of course, they are never lower and a subsequent de-rating of some of the stations amounted effectively to a further increase in capital costs. These facts are quoted in the book although they were not all known in 1965. Good influences such as the improvement in fuel element life are not quoted. It will be interesting at a later date to make a retrospective study of the cost of generating from these nuclear stations to compare with life-time figures from LWRs and with fossil fired stations. It is too early to say that the full truth has been revealed and that all is disaster.

In America, progress was through a series of subsidised demonstration reactors but this changed dramatically in 1963 when the Oyster Creek LWR power station was ordered. The quoted price was so low that it was regarded as being either heavily subsidised or a lost leader intended to sweep the market. Chapter 4 deals with the quick increase in costs between 1963 and 1966 but Oyster Creek did establish the LWR as probably the lowest cost system and it was accepted throughout the world, except in Britain, Canada and Russia.

In 1965, the advanced gas-cooled nuclear reactor (AGR) was expected to have higher initial costs than the LWR but lower operating costs as it was designed to produce steam at higher temperatures and pressures than the LWR and was matched to the large turbogenerators then being standardised on the CEGB system. Experience of the AGR was confined to the small demonstration reactor at Windscale, and the difficulties of extrapolation to larger sizes, later experienced also in the Magnox reactors, were not appreciated.

The reader of the book might infer that, in 1965, US experience was uniformly good, which it was not then nor always subsequently, whereas British experience was uniformly bad, which also was not true. It was in a different atmosphere from the one described by Duncan Burn that the Dungeness B assessment was made.

The CEGB procedures for tender assessments were well known within the Board and understood by those plant manufacturers who submitted tenders. It was the responsibility of specialist engineers, within the Board, to clarify any points of uncertainty in direct and confidential discussions with the plant manufacturers and, on the basis of their own calculations, to report at length on which submission best met the Board's needs In the case of nuclear power stations the procedure had become more complicated by the concept of 'turnkey' contracts which had been introduced and, in the particular case of Dungeness B and contrary to the view expressed in the book, by the exceptional amount of detailed design data provided It would have been impossible to proceed without very great help from the AEA, but the responsibility for a decision rested with the Board.

In chapter 10, it is stated explicitly that the assessment was biased and much of the blame is attached to the AEA, which is also accused of exerting excessive pressure on the CEGB. For example, on p163 it is said "in going AGR, the CEGB did so under the strongest pressure from the AEA" I cannot recall any meeting between the executive members of the CEGB and the members of the AEA at which pressure was even suggested and, if within the assessment team itself, anything was done which was unreasonable, I would have expected it to be resisted strenuously None was reported to the Board.

The conclusion was wrong for a very different reason. The extent of the extrapolation from the Windscale AGR to full scale was miscalculated and at Dungeness B, it was followed by some very bad engineering Part of the explanation for the miscalculation must be attributed to the fact that the CEGB had inadequate strength in nuclear matters to arrive at a conclusion contrary to that of the AEA or even to differ appreciably from their opinions. This was a matter for concern in 1965 and it still is.

The usual purchasing arrangement between a public utility and a manufacturer of plants outside the nuclear fields is dependent on a dialogue between two approximately equal sides This in the United States is roughly true for nuclear stations—also with little intervention from the national laboratories. In Britain, only fossil-fired power stations are treated in this way and the CEGB has an adequate engineering and R&D effort for a balanced discussion to take place, but in the nuclear field there is a single design and construction company—National Nuclear Corporation (NNC)—heavily criticised in the book) which is dependent on the AEA, as is the nuclear fuel company BNFL and, in addition, the main R&D resources lie with the AEA. It is very difficult to say where responsibility lies and the CEGB is not strong enough to deal adequately with the technical problems of new systems, in direct discussions with the manufacturers.

It is this centralisation of technical ability in the AEA which Duncan Burn also sees as contributing to the inability of Government sensibly to take technical decisions, and this is discussed at length in the final chapter of the book. He would wish to see a competitive arrangement, based on American experience, with the AEA and the Government largely withdrawn from the discussions between the generating boards and the manufacturers However, competition in the United States has reduced to only two firms to supply a large market Probably only one could survive here, although not necessarily the present NNC. The Government cannot withdraw entirely, for, as the main source of finance for the building of power stations and for the development of nuclear stations, it is entitled to ask the questions which any prudent banker would ask. And, especially in safety matters but elsewhere, too, there are political issues to consider.

The AEA position, as virtually the sole repository of information, will be changed with the recent decision to abandon the steam-generating heavy water reactor in favour of LWR and the main relationship might become direct between the generating boards and the overseas suppliers of LWR stations. But will it change very much if the board's negotiations have to be with the NNC and if the NNC is not free from the AEA? Even if the change is made, then, will it persist for the next Fast Breeder Reactor (FBR) the authorisation for which is long overdue, if it is to be available at the end of the century? Ought the CEGB to be happy with a situation in which its own R&D effort is concentrated largely on the operational problems of its already purchased reactors when it is the generating boards who will need the FBR by the end of the century?

There are many other questions raised by Duncan Burn's analysis and one can only hope that the book will stimulate more urgent consideration of the answers needed. It is worth reading for this alone but there is much more that the reader will find interesting and provocative. The only serious criticism would be that the views expressed would have carried greater weight if they had been presented with greater balance and fairness Even this is perhaps excusable, as it is a short book in which to cover everything on a big subject. □

L. Rotherham was Assistant Controller and Director of Research at the UK Atomic Energy Authority's Industrial Group from 1950–58, and a Board Member of the UK Central Electricity Generating Board from 1958–69.

Futurologists examined

Eric Ashby

World Futures: The Great Debate. Edited by Christopher Freeman and Marie Jahoda. Pp. 416. (Martin Robertson: Oxford, 1978.) £15.

In 1973 the Science Policy Research Unit (SPRU) at the University of Sussex brought a refreshing voice of sanity into the overwrought and querulous debate about limits to growth. In a collection of essays called *Thinking About the Future,* H. S. D. Cole and his colleagues courteously and convincingly exposed the irresponsible prognostications of doom put out under the auspices of the Club of Rome. If SPRU had made no other contribution to contemporary thinking, this work alone would have justified their existence. The team has now produced a second book which, in my view, is even more important. It sets out to make a sober analysis of the views of 16 "major authors" who have published forecasts about the possible futures or fates awaiting the world as it moves into a phase of too many people, too little food, too dangerous energy, too attenuated mineral resources: Kahn, with his cocky optimism that all will come right (if you don't mind a few wars and some pretty extensive starvation) provided we stay on the path of *laissez faire* capitalism; Forrester, rattling the bones of a computer in the face of society; Schumacher, wistfully hoping that trans-national corporations will metamorphose into cosy little cottage industries; Heilbroner, who sees hope only in authoritarian government; Tinbergen, who believes that a genuine crisis will mobilise loyalty among people for the common good; Kosolapov, whose Marxist optimism brings him astonishingly close to the capitalist optimism of Kahn; and 10 others: Spengler, Ehrlich, Meadows, Dumont, Mesarovic, Leontief, Kaya, Herrara, Modrzhinskaya and Galtung.

Christopher Freeman and Marie Jahoda have organised their material in a bold and successful way. They propose that the beliefs of the 16 authors whose work they consider, and the beliefs of their own team (by no means unanimous) can be roughly—and they emphasis that it is roughly—classified into three categories· conservative, reformist and radical; each with a distinctive "world view" which sets boundaries to the assumptions each author makes about possible futures. Thus, the radical may regard revolution as a necessary prelude to a future equilibrium; the reformist would favour big, but incremental, changes; and the conservative would question whether the background needs to be changed at all. They then make an equally bold generalisation about possible futures. Wisely they eschew computer scenarios which, as they say (p70), "disguise rather than reveal the extent to which their results are dependent on their assumptions and data". Instead they propose four options high growth accompanied by a persistence of the present inequalities between richer and poorer nations; low growth, also accompanied by the present inequalities; high growth accompanied by a major and persistent narrowing of the gap between rich and poor; and low growth, also accompanied by a narrowing of the gap between rich and poor. They then examine, in impressive detail, the possible consequence of these four options as they are perceived by conservatives, reformists and radicals. It is a skillful discussion of the interaction of three different political attitudes on four different possible futures for society. It is a simple framework on which facts and ideas can be attached, quite the most useful one which has yet been designed. In her eloquent conclusion to the book, Marie Jahoda sets out its three aims: to make a contribution to forecasting methodology, to take a substantive position in the world futures debate, and to offer some ideas for considering deliberate actions and choices in the less distant future which may affect the fate of the next two or three generations. They are modest aims, and all three of them are achieved.

No one is competent to take part in this 'great debate' who has not mastered the relevant facts about forecasts for population, food energy and mineral resources. These facts are set out in four introductory chapters, which cover familiar ground, although some of the conclusions drawn from the facts will be challenged. No one will dispute the recklessness of making predictions about population: the estimates by the 16 futurologists differ by almost an order of magnitude! Over food supplies the authors conclude that the limiting factor is not the amount of food which can be produced; it is the lack of means to distribute the food equitably and the lack of money in the Third World to pay for food. This view will be disputed by some experts, although no one is likely to underestimate the disgraceful mismatch between butter mountains in one part of the world and starvation in another. Over mineral resources the authors are equally optimistic: there will be shortages of specific materials but—in their view—these shortages will not be likely to have a desperate impact on the quality of life.

The Achilles heel for a stable future, the authors say, is energy If there is not a sufficient supply, all else—food, industry and peace—is put at risk. If a sufficient supply is ensured by massive development of fast breeder reactors and the like, then other dangers which have had plenty of publicity—what to do with wastes, what to do with terrorists—take the place of the dangers due to insufficient energy. The authors favour a eclectic solution, with much more attention than there is at present being paid to energy from the sun, wind and tides. Again, some experts will challenge this view as unrealistic

Differences of opinion over the content of these chapters do not, however, affect the main argument of the book, which is that the first symptom of crisis over food, energy, or resources will not be physical shortages of these things: it will be social and political disturbances; and it is to these that emphasis should be given, and given now.

The important and original part of the book carries a set of sober speculations. The authors make a list of seventeen features which play a part in determining the quality of life of ordinary citizens The list includes intangibles, such as geopolitical issues, and tangibles, such as food, health, transport and energy; and they add to these features the two emotive words which have drawn men into war and revolution for millennia: liberty and equality. How would conservative, reformist and radical regimes act toward these emotive words and the seventeen parameters for the quality of life in conditions of high or low economic growth, and at greater or lesser levels of equality? Into this matrix of three world views × four conditions of growth and equality × seventeen features contributing to quality of life (204 in all) the authors pack their conjectures for possible futures The matrix covers thirty-four pages; it contains, in my view, more practical common sense (which is the attribute which matters most in the Civil Service or in Government), and more credible assumptions (which are the most important ingredients for sound economic advice) than I have read in any other document about forecasting. Of course, it makes sombre reading. High economic growth continued indefinitely leads to the absurdity of all unconstrained exponentials. Low economic growth continued indefinitely leads to frustrated expectations, and these in turn lead to conflict. Perpetuation of the present inequalities between rich and poor nations are a recipe for revolution and war. Reduction of present inequalities — even with some growth — makes demands for sacrifices from affluent nations which, so far, have met with a derisory response. (The world's annual expenditure on armaments is some 15 times more than the aid from affluent to third world countries.) Struggle, turmoil, revolution, stagnation: these are words which appear unpleasantly often in the matrix

But the SPRU team, despite ideological differences among them, do come to a firm conclusion Of the four options they propose, the one which offers the least dangerous prospect for mankind is the scenario for high growth and more equality: The conservative would hope to attain this by creating a larger cake of wealth so that poor nations could share it: the reformist would expect affluent countries to react only under threat to world stability, and then, no doubt dragging their feet, to make sacrifices so that poorer countries could 'leapfrog' into appropriate levels of industrialisation. The radical would rely on the seizure of political power by the workers, followed by a gesture of altruism (not apparent among workers at present) toward their poorer brothers in the third world.

The outstanding practical conclusion from the analysis is that the absolutely top priority, the need which should take precedence over all other needs, is to narrow the gap between rich and poor, both within and between nations. This is not an original sentiment; but the authors of this book do not just offer it as a pious hope. They press the point: that the way to reduce inequality is to devote massive resources to problems to which the affluent nations are paying very little attention: research and development (for instance) on tropical agriculture and on intermediate technology. They stress, too, that innovation is needed far more urgently in political institutions than in technology Anyone who reflects on the problems which lie ahead is brought up against the ugly prospect that the problems may not be soluble under a pluralistic democratic government. The alternatives are one or other version of authoritarianism. Is it practicable to devise an authoritarian pattern of government which requires obedience over principles (such as is already demanded by the Highway Code in the UK) but leaves individuals freedom (as motorists in the UK indeed have) to exercise their liberty (for example, when driving, provided they comply with the code)?

The high level of generalisation in this book constitutes its strength. Indeed, the authors themselves repeatedly remind the reader of the futility of specific technology assessment. Discontinuities make nonsense of such assessments. For instance, the National Resources Committee of the Department of the Interior in the USA made a thorough prediction of technological trends in 1937. They failed to forecast atomic energy, computers, radar, antibiotics, and World War II and its social consequences: no-one can blame them for these omissions, but this example, and scores of others like it, should make anyone sceptical about similar specific forecasts being made today. The paramount potential discontinuity, of course, is the finite possibility of a nuclear war. In a heroic chapter, the authors of this book tackle this problem too And it leads them to a sombre conclusion namely that a policy of disarmament among the great powers (which would entail a cessation of activities costing over $\$200 \times 10^9$

per annum) would itself precipitate a major social and economic upheaval.

In a world deluged with books, many busy people have to content themselves with reading the reviews and not the books themselves. It would be a great mistake to read only the reviews of this book. It is essential reading for anyone who wishes to participate in the debate about man's future with (as the authors put it) "informed imagination". □

Lord Ashby is a Fellow of Clare College, Cambridge, and Chancellor of the Queen's University, Belfast.

Détente and dissent

Robert Lewis

Soviet Science. By Z. A. Medvedev. Pp. 262 (Norton: New York; 1978.) $10.95.

ACCORDING to the 'blurb' on its cover, Zhores Medvedev's latest book is

> "a brief history of science in the Soviet Union with an emphasis on the interaction between scientific and technological developments and the political goals of the Soviet government".

However, the potential purchaser should not be misled by this statement, for as the author himself admits in his introduction, this is not a book for those who wish to have a short and reasonably comprehensive account of the development of science and science policy in the Soviet Union since 1917. Indeed the years between 1917 and 1953 are summarily dealt with in five short chapters and a little over fifty pages. Furthermore these chapters contain several errors. For example, the author states that in the years 1922–28 science was directed at the government level by the People's Commissariat of Education, when, in fact, this commissariat was only one among a number of government organs involved in science policy-making and was becoming less important in this field. These initial chapters are padding which make the manuscript of better length for a book and which the publishers presumably hope will increase its marketability.

The real heart of Medvedev's book is an essay on science in the USSR under Khrushchev and Brezhnev with particular emphasis on the two themes of *détente* and dissent. The author restates his view that *détente* has not been a consequence of Soviet military weakness and is not a necessary condition for the continuing development of Soviet military technology. On the other hand he considers that it has had important consequences for Soviet scientists working in non-secret fields, bringing an acceleration in the renewal of links between Soviet and world science which had begun under Khrushchev. More Soviet scientists have travelled abroad and more articles have been published in foreign journals; the maintenance of informal contacts has become easier. This increased contact has strengthened the feeling among Soviet scientists that they, like scientists elsewhere, are participants in a universal scientific culture which is independent of any particular set of national values. This degree of cultural autonomy, reinforced by the high prestige which scientists have in Soviet society, has put them in a special position. Indeed, the author reports a conversation which he had with Kapitsa in 1967 when the latter suggested that the function of the United States' Supreme Court and the British House of Lords as independent arbiters in constitutional problems seemed to be falling morally on the Academy of Sciences of the USSR. These special conditions surrounding Soviet science are seen as major factors in the relatively high frequency of political dissent amongst scientists. Medvedev reviews the policies adopted by the Soviet government and the Communist Party to counter dissent by such means as the exercising of closer control over the award of higher degrees and promotion with the aim of preventing dissidents rising higher in the scientific hierarchy; he is, however, sceptical of the effectiveness of such measures.

Thus, the Soviet government, as the author points out, is in a dilemma. On the one hand the future progress and prestige of Soviet science, to which the government attaches great value, depends on international contacts—a simple instance is that, as a result of lengthy publication lags in Soviet journals and of the inability of most other scientists to read Russian, publication abroad is likely to be needed in the vital matter in modern science of establishing priority. On the other hand the closer attachment of Soviet scientists to the international scientific community and its norms and values increases their degree of cultural autonomy and consequently the problems of political control. The author does not offer any firm prediction of the likely outcome of this clash of values, but he does foresee the possibility of future new waves of open dissent and that these could be stimulated rather than eliminated by attempts to control science and scientists more closely. □

Robert Lewis is Lecturer in Economic History at the University of Exeter, UK.

Soviet technical intelligentsia

Julian Cooper

Technology and Society under Lenin and Stalin: Origins of the Soviet Technical Intelligentsia, 1917–1941. By Kendall E. Bailes. (Princeton University Press: Princeton, New Jersey, and London, 1978.) £20.10.

THE role of engineers and other technical specialists in a society in transition between capitalism and socialism is a question which, for understandable reasons, received little attention in the writings of Marx and Engels. In a rarely cited letter of 1890, however, Engels wrote:

> "Of course we still have insufficient technicians, agronomists, engineers, chemists, architects, etc., but if the worst comes to the worst we can buy them for ourselves, just as the capitalists do, and if some of them turn out to be traitors (as will certainly be the case in that society) they will be punished for the edification of the others, so that they will appreciate that it is not in their own interests to rob us any more. But with the exception of these specialists, and also the school teachers, we can very well do without the rest of the 'educated'. For example, the current strong influx into the Party of penpushers and students brings all kinds of trouble in its wake, unless these gentlemen are kept within proper limits".

Lenin, in the years preceding the October Revolution, also found such 'gentlemen' troublesome, but immediately after the successful winning of power the Bolsheviks had to face up to the problem of retaining the desperately needed skills of the technical specialists, the overwhelming majority of whom were unsympathetic or actively hostile to the Soviet power. In his pioneering study, Kendall Bailes, associate professor of history at the University of California at Irvine, is concerned with the fate of these engineers and technicians and the contradictory process by which a new technical intelligentsia, in the main loyal to Soviet society, was formed. The relationships between the technical intelligentsia and other social groups are

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explored and an assessment made of its place and role in the political system during the formative years of the modern Soviet state.

As Bailes lucidly relates, in the early years under Lenin's insistence and in the face of opposition within the Party the Bolsheviks did indeed 'buy' for themselves the services of the bourgeois technical specialists, both by ensuring them relatively privileged material conditions during the lean years of civil war and famine, and also by providing improved opportunities for applying their professional skills In the very first years of the revolution a number of important new applied research institutes were founded and many engineers, though unsympathetic to the regime, were involved in the drawing up of the famous GOELRO plan for electrification of 1920.

At this time, as Engels predicted, 'traitors' were punished for the edification of their colleagues. By this carrot and stick approach the Party succeeded in winning the majority of the old technical specialists to work for the Soviet power, and some forsook their political antipathy or neutrality and joined the Communist Party. From 1928 to the summer of 1931, at the time of the adoption and initial implementation of the ambitious first five-year plan and the associated struggle against the right-wing opposition led by Bukharin, a section of the old technical intelligentsia came under attack, accused of 'wrecking' activities. These events are described in detail by Bailes, who argues with originality and plausibility that the Party leadership around Stalin feared the growth of a 'technocracy' movement which, like its counterpart in the United States at that time, sought to elevate the engineers as a professional group to a position of political influence and power.

In order to break the country's dependence on technical skills without socialist commitment, the Party launched a large-scale programme for the education of a new generation of Soviet engineers, the majority of whom were of working class origin. Bailes devotes considerable attention to the formation of this new technical intelligentsia and chronicles its expansion and mounting influence during the 1930s. In the purges the technical specialists, like other professional groups, suffered greatly, although Bailes is probably correct in suggesting that engineers and technicians had a higher survival rate than, say, the industrial managers. Even after the worst waves of repression many representatives of the old technical intelligentsia continued to occupy posts of authority in industry and higher education. Before and during the purges Stalin and other Party leaders constantly stressed that specialists had to be politically conscious and committed, and it is notable that by 1937 there were almost 50,000 Party members with higher technical education, compared with a mere 138 graduate engineer Party members in 1928

The rapid economic and social development of the pre-War decade was accompanied by a radical transformation of the political status of the engineers they started as critical outsiders and ended as a significant group within the Party and government. Between 1939 and 1941 no less than 70% of new Party members came from the ranks of the administrative and technical intelligentsia. As Bailes observes, many of the members of the present-day Party Central Committee and Politbureau, including Brezhnev himself, are representatives of this new generation of technical specialists who embarked on their careers in the turbulent pre-War years.

In discussing the origins of the Soviet technical intelligentsia (throughout inelegantly and inaccurately termed the 'technostructure'), Bailes draws on an impressive range of sources, both Soviet and Western, and presents much new interesting material Rejecting both the totalitarian and the more recently fashionable interest groups models of Soviet politics, the author adopts a more flexible approach, placing greater emphasis on the interaction of social forces making for cohesion and conflict than is usual in discussions of the Soviet Union in the 1930s. This leads Bailes to analyse political developments between 1931 and 1937 in terms of a growing tension between the industrial managers and technical specialists and their spokesmen in the Party leadership on the one hand, and the group of 'Stalinists' (not satisfactorily defined) on the other, with the planning process as the focus of discord. But this interpretation, central to the author's discussion of the purges, is not wholly convincing The planning process is seen one-sidedly in terms of the setting of output targets, to the neglect of problems of the allocation of inputs, which at times produced quite different conflicts of interests. In general, the author's grasp of economic and, in particular, technical matters is not as sure as his understanding of the processes of social and political changes, although in analysing the latter a debatable definition of class is used, according to which manual and non-manual workers, including the entire technical intelligentsia, form two distinct classes

With a work of such impressive thoroughness and length it is perhaps unreasonable to point to omissions, but one would have welcomed more consideration of the substantive technical issues which were debated by the engineers, often with great passion, during the years of rapid industrialisation—for example, the relative merits of American mass-production engineering technology as opposed to the traditional Western European methods. There is also insufficient consideration of the important contribution of foreign technical specialists (over 9,000 were working in the USSR in the autumn of 1932) and a related tendency to divorce Soviet internal developments from events in the then increasingly hostile outside world: it could be argued, for example, that the moves in the summer of 1931 to improve relations with the old technical intelligentsia were not unconnected with the measures in May to reduce the level of reliance on foreign technical assistance.

Kendall Bailes has provided an important contribution to the steadily growing body of literature on the history of Soviet society which seeks to take a fresh approach based on serious original research, rejecting the crudities and prejudices sadly prevalent during the Cold War years ☐

Julian Cooper is a Research Fellow at the Centre for Russian and East European Studies, University of Birmingham, UK.

History of integrated circuits

Andrew Holmes-Siedle

Revolution in Miniature: The History and Impact of Semiconductor Electronics. By E. Braun and S. MacDonald. Pp. 231. (Cambridge University Press: Cambridge and London, 1978.) £8.50.

"AN integrated circuit is a truly awe-inspiring achievement of human inventiveness". So say the authors of this book Those working on silicon technology, who know what effort and physical insight is packed into those minute structures, will not find it difficult to agree with this statement. For those not so privileged, the integrated circuit is likely to inspire puzzlement rather than awe. The structures concerned consist merely of microscopic modifications on the surface of a silicon chip. They usually lie sealed in anonymous little ceramic or plastic blocks and these themselves are commonly shut away inside the case of a calculator, television set or satellite. Hardly stirring stuff for the average man. How can one bring home to the layman the undoubted revolution which they are bringing about in industry and the home?

The authors of this book, who are members of the Technology Policy Unit at the University of Aston in Birmingham, have made an attempt to view the history of the integrated circuit as a whole and bring a little of its flavour across to the intelligent reader. They have tried to add a little life to that formidable array of technological fact in several ways: first, by conducting interviews with some of the engineers and physicists involved and quoting parts verbatim—this is very successful; and second, by adopting a loose form of comment which one can only call journalese ("for a fraction of the cost of one week's housekeeping, one can have access to any number's square root"). The results are sometimes fascinating but often baffling, especially when sweeping statements are made without support and the references, when one turns to them, are more often to *Business Week* than to the original literature. Perhaps this is the style of theses in technology policy but it is galling to a serious reader who is reaching for his pencil in anticipation of an original historical reference which he can follow up. Perhaps this is asking too much, however, of a book which the authors direct to the "intelligent lay-reader" Nevertheless, there are at least 600 references given. This makes the work a curious mixture of academic study and "pop history".

The opening chapters of the book are well worth reading, being an enjoyable and brief account of the history of the invention of the transistor, with suitable comments on the influence of science, commerce, the military and the upheavals caused by World War II. The hothouse atmosphere of Bell Laboratories is well created and the subsequent commercial spread of the transistor is dealt with in fairly conventional narrative form. The authors avoid any diversions into technical explanation, possibly leaving a little too much for the general reader to pick up elsewhere. It is noteworthy that the only line-drawings in this book are financial charts. How many scientists could write a book without recourse to technical sketches?

In this section, historical balance is lacking in at least one respect: the invention of the metal-oxide-semiconductor (MOS) transistor by Hofstein in 1962 is passed over too lightly. Although the events of the late 1940s at Bell are exhaustively documented (at least 60 references are quoted including dozens of interviews), the inventor of the MOS device is neither named nor interviewed. The term "MOS" is only mentioned a few times in the whole book and this can be viewed as a serious lack of balance, because the ability to manufacture large-scale integrated circuits cheaply from MOS elements has been the main driving force of the revolutions of the 1970s. It is also predicted by others that MOS circuits will dominate microelectronics in the 1980s. In fact, the history of the Intel company, the manufacturer of the first MOS microprocessor, is probably as important as that of Bell, but it is not described except as part of a more general description of Silicon Valley.

The later chapters of the book bring the reader up to date concerning the invention of the integrated circuit and the immense expansion of uses which came with this step. More space is given in this section to business considerations, such as figures for the growth of markets and the pricing of components. It is likely that the technologist will find this more interesting than will the lay-reader It is also here that the very speed of technological progress which all commentators emphasise seems already to have dated the book. Electronic games, Viewdata and the charge-coupled device are not mentioned, and the word "microprocessor" is not even listed in the index. The splendid compatibility between the integrated circuit and the satellite is not noted and Space is, in fact, only mentioned as part of an "awesome list" of weapons. These, the authors point out, are "packed with electronics from top to bottom". It is in this section that oral history techniques come into their own The interviews give the right tang of "entrepreneurialism" (*sic*) flavoured with physics which characterises the microelectronics industry in the USA. The authors should certainly persevere with this method.

The concluding chapter presents "Reflections of an Electronic Age". From authors who have performed a social analysis of the past three decades of electronics, one expects some penetrating statements covering future applications and the future cultural influences of microelectronics. This the authors attempt to do but the statements too often dissolve into generalisations that are far from penetrating. It is not too much to ask even a popular history book to be penetrating.

We badly need help in understanding the wide social impact of microelectronics. This book may help many by its factual survey but, for this reader, it provided no more than the faintest glimmer of fresh understanding. ☐

Andrew Holmes-Siedle is a Consultant to the Physics Department of the Fulmer Research Institute, Slough, UK.

What use is astronomy?

A. J. Meadows

Man and the Stars. By Hanbury Brown Pp.182. (Oxford University Press/Clarendon: Oxford and New York, 1978) £5.95, $14 95.

PROFESSOR Hanbury Brown explains in the preface how he came to write this book:

> For fifteen years I have run an astronomical observatory in the Australian Bush . . . In that time I must have shown the place to thousands of visitors and tried to explain what we were doing and why . . However, there was one particular question which always worried me because I couldn't answer it satisfactorily in the time available, usually only a few minutes before the coach left. It was often asked tentatively, away from the main group of visitors, as though it might be silly or, perhaps, impolite It was always the same basic question—'This work may be competent and interesting, but is it any use?'".
>
> I found it comparatively easy, given a few minutes, to persuade people that our work was relevant to the general study of astronomy, but I have never been able to abbreviate, to my satisfaction, the arguments that astronomy is an integral part of science and that science is an essential part of our civilization. And so, as the coaches vanished in a cloud of red dust, I was often left wondering what the visitors really thought of our observatory. Many of them, I suspect, felt much the same as they would have done after a tour of a monastery—interesting maybe, but a useless way to spend one's life!

What this book sets out to do, therefore, is to try and sketch out Professor Hanbury Brown's own answer to the question—what use is astronomy? It is a question that should engage the attention not simply of astronomers, but of all pure scientists. Most of us have our salaries, our accommodation and our equipment provided by the state—that is, ultimately, by everybody else in the community How can we justify this expenditure to them?

Here, for astronomy, we are offered answers under five headings: stars and the calendar, stars and time, stars and navigation; stars and science, stars and man. (I am not altogether clear why the title is *Man and the Stars*, even though the chapter headings run in the opposite direction Perhaps it is to remind us that astronomy could not be practised if either of the two components 'man' or 'stars' were missing.)

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Second Edition

THE EARTH'S TECTONOSPHERE

ITS PAST DEVELOPMENT AND PRESENT BEHAVIOUR

J. H. TATSCH

We are pleased to announce that the first (1972) edition of *The Earth's Tectonosphere* has been completely revised. The new edition updates the results of our continuing research to determine the most probable geometrical, mechanical, thermal, and chemical aspects of the origin, evolution, and present behaviour of the Earth's upper 1000 kilometers. Included are about 80 of John C. Holden's inimitable drawings.

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Available directly from the publisher or through book dealers in all parts of the world. Hard cover, 6×9 inches, 468 pages. Table of contents, line drawings, maps, bibliography, index. $30 LC: 74-78917. ISBN 0-912890-03-7. 1977.

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The purpose behind each of these headings is to emphasise how the development of astronomy and the growth of other human activities and thought interact. From this viewpoint, the first three chapters should really be read together They recount how man down the centuries has used astronomy as a means of fixing his position in time and space. This ability is obviously fundamental to the existence of civilisation. Agriculture and religion needed a viable calendar; organisation of the working day required good time-keeping, travel and, therefore, commerce over any distance demanded accurate navigation. The fourth chapter tackles a different aspect of astronomy: its role in the advancement of basic science and, in particular, physics. The final chapter looks at the much more diffuse and difficult question of the influence astronomy has had on the way we interpret and experience the world around us.

We are provided with a well written and lucidly argued case for the importance of astronomy, but I feel that somehow, for much of the book, the author actually avoids the main point. The real question is not whether astronomy had immediate relevance to everyday life in times past, but whether it has such relevance today. Already in the eighteenth century, astronomy was ceasing to be of practical concern to more than a few people. Thus, the astronomer in Samuel Johnson's *Rasselas* says that he has devoted his life to the wrong subject, because the knowledge he has acquired is of little use to mankind. Of course, astronomy still has some practical uses today. Some years ago I was telephoned by a major British company, who required a quick astronomical method of checking some alignments in their surveying operations. I suggested a method which was adopted—so, is this not a clear example of a practical use of astronomy? (As I forgot to ask for a consultancy fee, it unfortunately does not simultaneously demonstrate the practical use of astronomers.) But the concepts behind my proposal had been known for centuries. The problem is that the ideas most astronomers are concerned with today seldom, if ever, have relevance to everyday affairs. I find the counter-examples that Professor Hanbury Brown suggests distinctly unconvincing. For example, he mentions the importance of astronomy for space navigation. But this simply raises the question—what is the current utility of space navigation? The only space activities which certainly have a value for everyday life are those concerned with Earth applications, and the satellites involved in these hardly require space navigation.

It is worth laying considerable stress on astronomy's lack of immediate utility because it distinguishes astronomy from other major divisions of science. The others can all claim to have something to contribute towards the material interests of human beings; but astronomy has declined over the past few centuries from being a highly useful subject to being almost useless. Nor has astronomy provided much basic information to other areas of science. Rather, throughout the twentieth century, astronomy has taken more from other disciplines than it has given to them. Yet astronomical research has not declined over this period. On the contrary, state support for astronomy is now provided on a larger scale throughout the world than would have been imagined possible a century ago.

If we seek a reason for this funding it must derive then from the importance of astronomy for our view of the world. This has continued to be recognised long after the practical value of astronomy declined. Thomas Huxley, presumably unbiassed as a biologist, claimed that astronomy is the science "which of all sciences has filled men's minds with general ideas of a character most foreign to their daily experience, and has, more than any other, rendered it impossible for them to accept the beliefs of their fathers". As long as this sort of claim can be made for astronomy, or for any other branch of pure science, it can surely hope to receive financial support. But there is a proviso. For astronomy to affect our view of the world, its advances must be made available and understandable to people who are not astronomers. Professor Hanbury Brown therefore concludes his book with a plea for the proper popularisation of science. He is to be congratulated on practising what he preaches in such a readable way. □

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Technological day-dreams

R. L. F. Boyd

Spaceships of the Mind. By Nigel Calder. Pp.144. (British Broadcasting Corporation: London; Viking: New York, 1978.) £6.50; $14.95.

WHEN a project in space science or technology is undertaken in the real world, the lives and reputations of real people are at risk. Real public money is spent for which there are many competing calls: many of them are less worthy, but some may have stronger claims on resources. Those involved in the decisions and execution of the projects are usually alert to the glare of publicity surrounding their activities and the incalculable consequences of failure. The long road to realisation involves a succession of studies followed by detailed scrutiny and by battles for authority to proceed, which may be lost or won. A typical sequence involves Mission Definition, Pre-feasibility Study, Feasibility Study. These are sometimes called Phase A, may cost several per cent of the final project and usually have a significantly less than even chance of surviving for further work.

The next phase — Phase B — the Design Definition, involves a hard and disciplined engineering grind in which 'state-of-the-art' parts of the project are carried through to a point of readiness when a "metal-cutting" realisation of the project — Phase C — can be or perhaps cannot be entered upon. Any parts of the project requiring research and/or development will have been identified, assessed and costed. Some limited experimental work such as determining the emissivity/absorptivity of a particular surface material or its deterioration by solar ultraviolet radiation may have been undertaken. Exceptionally the procurement of long lead items not representing a major part of the cost may have been put in hand. By this time 10% or so of the total cost to launch will have been committed. That cost will have been established to within about 10% and a decision whether or not to proceed must be taken.

Phase C, the production phase, is a long agony of finding out how difficult things are in practice; fighting specification deviations (one firm supplied me with stainless steel screws made, we discovered in the final magnetic survey of an Orbiting Solar Observatory, from "the nearest alloy to the one specified which was available"; they were excellent in all respects except the one that mattered most; they were magnetic); fighting safety problems real and in the minds of ill informed safety officers; testing, going back to the drawing board, re-testing, accepting or rejecting compromises and so on.

Spaceships of the Mind make no such demands on reality but like other hallucinations are probably best regarded as a (bad?) dream. I would have settled for science fiction but the necessary imagination and drama are absent. Calder's previous book *The Key to the Universe* (but not the television production) was, in my view, a brilliant service to science. It showed to the intelligent layman such coherence in our understanding of the mystery of being as could be shown and my only quarrel was with the suggestion that there is likely to be a key to so much that could be grasped in any ultimate way by the limited system of neurones in the human brain.

Unfortunately this latest book (mainly about space colonisation) fails to convey the nature of the process by which technical problems are specified and solved, thus obscuring the gap between the dream and reality. I have theoretical astrophysicists on my staff but when I want to know what can or cannot be done for a projected payload I go to my engineers.

Calder goes to distinguished scientists of which the late J. D. Bernal is the prototype. He talked to John Lewis a cosmic chemist, to Freeman Dyson an eminent theoretical physicist, to Sol Spiegelman a distinguished molecular biologist, to Stephen Cheston an historian, and to many others of comparable distinction and similar qualifications. He did talk to some engineers. The late Val Cleaver was one of the greatest, and he was inclined to think that a "distinguished physicist" like O'Neill "ought to know better" than to suggest space colonisation on a time scale of decades. Theordore Taylor was a rather different engineer whose ideas entitled by Calder "The Santa Claus Machine" seem to me as far from any realistic materials science as that childhood myth. I cannot believe that justice(?) has been done to this former Princeton professor. I feel the same about Henry Kolm and his "mass driver"—an electromagnetic gun for ejecting pieces of asteroids or the Moon or what-have-you either for reaction propulsion or as a kind of space parcel post. These engineering essays carry no conviction because they are presented with no calculations. This is a disservice. Above all else, physical science and engineering is numerate. If this lesson were learnt at school we would be spared the correspondence from cranks who invent this or discover the explanation of that, when a sum on a postage stamp would expose the nonsense.

On the credit side, Calder does devote some attention to the Earth which in all conscience needs it more surely than the rest of the galaxy; he shows some recognition that ethical problems of humanity may overwhelm the day-dreams and he makes it clear that his sample of prophets has been strongly selected to "test the hypothesis that recent advances in scientific knowledge ought to have stirred constructive thoughts". The hypothesis is verified. What other thoughts will be stirred for good or ill I cannot guess. If you are only going to buy one book buy *The Key to the Universe*. If you are going to buy two let me commend a story Dingle once told before a lecture by Eddington on his more recondite and less certain speculations. A man went into a restaurant and asked for a boiled egg and a kind word. The waitress brought the egg and turned to leave the table. He reminded her he wanted a kind word. "Don't eat it", she said. ☐

R. L. F. Boyd is Director of the Mullard Space Science Laboratory, University College, London, UK.

Rebel without a cause

Michael Rowan-Robinson

Scientists Confront Velikovsky. Edited by Donald Goldsmith. (Cornell University Press: Ithaca, New York, and London, 1978.) $8.95; £5.95. *Velikovsky and Establishment Science.* Edited by L. M. Greenberg. (Kronos: Glassboro, New Jersey, 1978.) $9.95.

IN 1950 a remarkable book by a Russian born psychiatrist, Immanuel Velikovsky, who had lived in the US since 1939, was published. The book, *Worlds in Collision*, argued that the biblical account of the Israelites' flight from Egypt, together with numerous other ancient texts from cultures all over the world, carried the record of great natural catastrophes. Venus, ejected as a comet from the planet Jupiter, passed close to the Earth, causing earthquakes, volcanoes, floods and plagues, then dislodged Mars from its orbit onto a near collision course with Earth, before settling in its present circular orbit. The book aroused fury amongst certain members of the astronomical establishment. Efforts were made to prevent publication of the book, and to persuade astronomers to boycott the publishers.

Gradually the furore subsided. The book has been popular with the public but has for the most part been ignored by astronomers. Velikovsky is an excellent writer and if he had taken the trouble to learn what is known in astronomy and geophysics, he would have made a wonderful populariser of the field. As it is the book is of little scientific interest. None of the ideas are developed in any quantitative detail. The "predictions" are of the type: Venus is hot, Jupiter will be "a source of radio noises", the polar caps of Mars are "of the nature of carbon", argon and neon will be present on Mars "in rich amounts". Velikovsky and his supporters make great play of the cases where these "predictions" fit subsequent observations, but no effort is made to confront the theory as a whole with what is known.

In the early 1970s amid the growing popular interest, particularly in the US, in offbeat ideas and cults like parapsychology, the occult, astrology, and UFOs, Velikovsky became something of a guru. Two publications, *Pensée* and *Kronos* were devoted to his ideas. The young and rebellious flocked to his banner. Once again the establishment became alarmed. At the February 1974 meeting of the AAAS in San Francisco, a symposium was held at which four scientists tried to confront Velikovsky with the brick wall of facts which his theory must run into. One eye-witness told me he felt sorry for the way this old man was being utterly demolished by these arguments. Yet in his reply, Velikovsky steered his way round the wall to the satisfaction of himself and his vocal supporters.

It is unfortunate that *Scientists confront Velikovsky* contains only the arguments against Velikovsky, for it reads like Hamlet without the Prince. Velikovsky's contributions, together with nine other articles by his supporters, appear in a separate book, *Velikovsky and Establishment Science*. The reason for this fragmentation of what ought to have been the *Velikovsky Symposium Proceedings* is that Velikovsky asked for more space to answer his tormentors than the editor was willing to allow. To me this seems a piece of unnecessary pettiness. In the event Velikovsky's contributions amount to rather fewer words than those of his principal inquisitor, Carl Sagan.

It has to be said that Velikovsky faced his ordeal with considerable dignity, both at the symposium itself and in his published articles. This is not always true of those whom the scientific establishment rejects and despises. In some, like Herbert Dingle in *Science at the Crossroads*, the bitterness of their treatment becomes more important than the scientific case they are trying to make. This type of attitude is prevalent in many of Velikovsky's supporters. The main object of their hate is Sagan and they have worked very hard to ferret his more eccentric speculations out of the literature, with some success. Why should Sagan be allowed to speculate about life on Mars, extraterrestial civilisations and "galumping beasts" out there, and then ridicule

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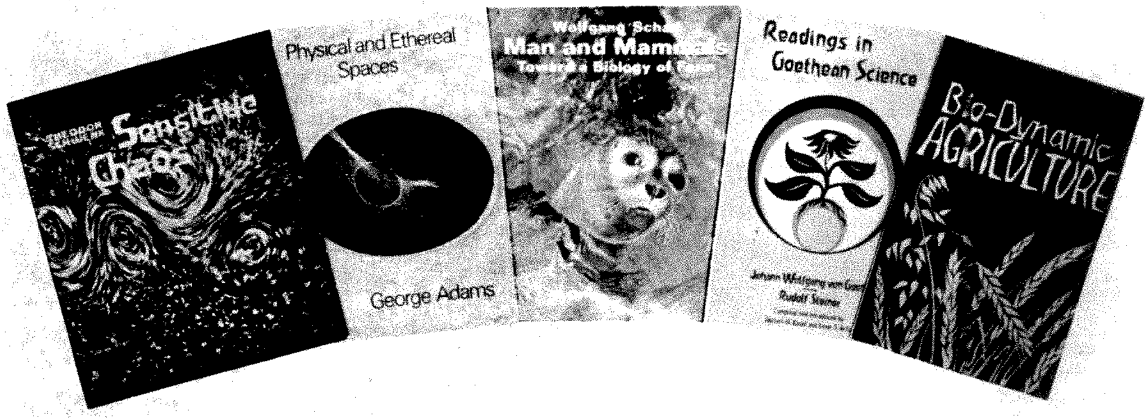

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Fact or Fallacy? M. Bowden

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NEANDERTHAL MAN. The evidence that these were true men suffering from rickets, arthritis and syphilis.

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RAMA PITHECUS. Fossil evidence only consists of jaws and teeth. L. S. B. Leakeys devastating letter regarding their classification.

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Velikovsky's notion of fly larvae in the tails of comets? More generally, why is it OK for Hoyle and Wickramasinghe to speculate about world-wide epidemics brought by cometary dust, and for Sagan to speculate about the evolution of human intelligence, but not OK for Velikovsky to speculate about astronomy?

The crucial point, to be borne in mind by all weekend cosmologists, is that a demonstrated awareness of what is known and what current ideas are is a prerequisite to being listened to seriously by scientists. The more you have demonstrated that you know, the more your speculations will be listened to. If you are Hoyle or Ryle, your views on energy production will be listened to with sympathy. If you are Einstein, your views will even be sought on the international political scene.

How good a demolition job did Sagan *et al.* do on Velikovskianism? Although some of them, particularly Sagan and Huber, obviously put a lot of work into preparing their contributions, the result is not altogether satisfying Rather too many weak arguments, niggling at peripheral issues, appear in these pages The most solidly argued case is that of David Morrison (not actually a symposium participant) who doesn't bother to chase every hare Velikovsky starts. He concentrates on the solid bedrock of facts that Velikovsky is inconsistent with; the huge difference in composition between Venus and Jupiter, and between the allegedly interchanged atmospheres of Venus, Mars and Earth; the evidence that the era of major cratering on Mercury and the moon ended 4×10^9 yr ago, rather than 3,000 yr ago as Velikosky believes; the evidence that the moon has never been molten, so that craters cannot have formed by bubbling, the evidence from the craters of Venus that it cannot have been torn from Jupiter recently, and the absence of evidence for residual excess heat in Venus and Mars

Sagan has additional arguments involving the difficulty of ejecting Venus from Jupiter; the low probability of cometary encounters with Earth, the difficulty of restarting the Earth's rotation once it has been stopped; the problem of circularising the orbit of Venus; and the total mass of manna required to feed the children of Israel for 40 years. Apart from the manna question, which I find a bit of a red herring, these difficulties do not seem to me insuperable given the initial catastrophist framework Similarly Huber's demonstration that the ancient Babylonian Ammizaduga cuneiform tablets are consistent with the modern orbit of Venus is fascinating, but hardly compelling evidence against Veliovsky in the light of the data massage, not to say downright resuscitation, required.

The arguments of *'Scientists confront Velikovsky'*, though of variable quality, are overwhelmingly strong enough in totality to reject Velikovsky's scenario. Why then do the young and rebellious, not to mention a few professors of history, philosophy and anthropology, follow Velikovsky? Asimov, in an entertaining forward to the anti-Velikovsky book, suggests: "Supply the public with something amusing, that sounds scholarly, and that supports an idea it wants to believe (in this case the literal truth of the Bible), and surely you need nothing more."

Does anything then remain of Velikovskianism? What is the basic core of his idea? That a comet passed near the Earth in classical times causing chaos. Suppose a massive long period comet were first noticed towards Jupiter and then appeared as a bright evening or mornning star before brushing past the Earth Would not the ancient myths then speak of Venus, daughter of Jupiter, bringing disaster? Stripping Velikovsky's scenario of the direct association with Venus and Jupiter eliminates almost all the refutations offered against him It also, of course, eliminates almost all Velikovsky's astronomical "predictions". What would remain is his evidence from the ancient texts, easily the most interesting part of *Worlds in Collision*. Can such a scenario be refuted? And if it were one day proved correct, would we say that this curmudgeonly old rebel had a cause after all? ☐

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Ptolemy in the dock

D. T. Whiteside

The Crime of Claudius Ptolemy By R. Newton. Pp.411. (Johns Hopkins University Press Baltimore and London, 1978.) £15.75.

"THIS is the story of a scientific crime", begins the author, "committed by a scientist against his fellow scientists, a betrayal of the ethics and integrity of his profession that has forever deprived mankind of fundamental information about an important area of astronomy and history" And he concludes his summing-up for the prosecution "All of his own observations that Ptolemy uses in the *Syntaxis* are fraudulent, so far as we can test them . His work is riddled with theoretical errors and with failures of comprehension . His models of the moon and Mercury conflict violently with elementary observation . . The *Syntaxis* has done more damage to astronomy than any other work ever written, and astronomy would be better off if it had never existed " In between, for almost four hundred pages, comes a relentless attack on the character and achievement of "the most successful fraud in the history of science" such as would do any cross-examining QC in the Old Bailey proud

Shifty-faced Ptolemy in the dock is found morally guilty of systematically falsifying past records for his own ends and left with only the odd one of his own observations of the heavenly motions which "may be genuine" (never, of course, the charity of "may be false".) He is not allowed credit even for his bissextile equant model of circular motion for the outer planets and Venus—that on which alone a near-perfect theory of all the solar planets may be founded, and which even Newton must allow to be "indeed . . a contribution to astronomy" "There is no evidence", he thunders, "that [its discovery] was made by him . . and Ptolemy lessens its value considerably by his inaccurate use of it" What a dullard lot succeeding astronomers have been—Copernicus especially (on whose debilities Newton is almost equally severe) —never for the best part of the next two millennia to have suspected any of this! And the wise juror waits patiently for the counsel for the defence to present his counter-case

From Newton he will get barely a glimmer of what that might be. Nor is here, in a short review, the place to specify with full subtlety and technicality of argument how time and again he fails to set out fairly the difficulties, theoretical and observational, which Ptolemy had to face in creating his synthesis, imperfect as it was, of existing astronomical knowledge—this through a previous analysis of data, 'truly' reduced from observed phenomena or confected *ad hoc* as they might severally have been, at which we can for the most part make only reasoned conjecture by re-analysing the finished synthesis itself as we alone have it

The modern fast computer allows us, it is true, freely to apply one powerful independent touchstone as to how the Sun, the Moon and the night sky would have appeared to the ancient observer (if we make certain minimal, seemingly well-founded, assumptions about the stability of the 'fixed' stars and the unchanged basic motions, secular and periodic, of the Sun, Moon and planets over the past several thousand years): that of extrapolating back from our modern accepted theories of such apparent motion. And on this test Newton bases the numerous charges of fraudulence in observation which he hurls at Ptolemy. Yet one must be very careful in interpreting divergences between 'observed' values and the predicted past event as it 'must' have been seen.

Modern theories are too exact for the historian's purpose, too well corrected for all manner of disturbing factors to afford a proper basis of comparison. Moesgaard and others have indicated that many of the past recorded sightings (those of lunar eclipses above all, on which depended in classical times all precise geometrical modellings of the Moon's motion) were themselves available to Ptolemy in a version already reduced to norm according to a variety of simple interpretative models about which he had no sure knowledge. And so to some degree it was no sin for him to hone their roughnesses. As for the raw observations themselves, there is no need to lay heavy stress on the gross inaccuracies and imprecisions which beset all sightings by the naked eye using only the most primitive of mechanical aids: at best the Greek astronomers could call on the help of an armillary sphere and a calibrated ring-dial ('astrolabe'), and their only exact means of timing was to use the diurnal motion of the Earth in shooting the Sun and the stars.

All of this Newton takes into account, though without emphasising the size of the errors—15′ of arc is nothing—which swiftly agglomerate. There are other less obvious sources of systematic error in taking sightings from our moving Earth which he does not mention at all, such as aberration, and above all atmospheric refraction and—complementary to this as it was measured—the observer's horizontal parallax. (In the late sixteenth century Brahe, the first to give tables for aerial refraction, still set upon the parallax a maximum value of 3′, some 20 times too large; and Ptolemy must have made reduction to the Sun by one much greater than this.)

When Mercury was only ever glimpsed near to maximum elongation, around dawn or dusk inevitably when the incoming light ray was bent up to 40′ from straight, we ought perhaps to admire Ptolemy for his bravery in putting his head on the chopping block with any attempted model of the apparent motion of that highly eccentric and perturbed closest satellite of the Sun. And who in honesty can be harsh with him for not more exactly predicting the orbit and changing apparent size of our own Moon when the problem defeated the combined efforts of all—even Newton's seventeenth-century surnamesake (who blandly changed the sign of one inequality in his lunar theory when it suited him)—before Euler and Mayer only two centuries ago?

CL PTOLOMÆUS ALEXANDRINUS Mathematicus
Nasc. Alexandriæ
Ao.
obyt
Ao.
Sustinuit caelos humeros fortissimus Atlas,
Incubat ast humeris terra polusque tuis

Claudius Ptolemy

From an engraving by De Brij, about 1648

Not to say that Ptolemy's crank mechanisms for duplicating the motions of both these bodies do not, in their structure as well as their given parameters, rest on readily shakeable foundations. Gingerich, and Delambre long before him, have already shown us some of Ptolemy's eye-deceiving Potemkinist fabrications here. And Newton evinces all the puritanical zeal of the determined iconoclast in endeavouring to prove in the body of his present book that no more depth of reality underlies the rest of the *Syntaxis;* that this "Almagest" is the "greatest" only in the scale and elaborateness of its astronomical *trompe-l'oeil.* In his pages, to be fair, anyone with a passably numerate intelligence and a reasonable degree of historical understanding—this is not a book for the beginner, whatever Newton may say—will find much of the fine web of his explanatory analyses and reconstructions of Ptolemy's mode of thought and composition extremely persuasive, if not everywhere wholly convincing. Again, would there were room here to say more. It makes a good companion to Pedersen's recent exegesis from a different, more conservative viewpoint in his *Survey of the Almagest,* and to the wider precisions of Neugebauer's *History of Ancient Mathematical Astronomy.* A pity that Newton does not write his controversial account of the *Syntaxis* with a little more of their calmness and humility. One grows weary with the rantings of a pulpit-thumper who interminably moralises and chastises.

In the final count I remain unclear as to exactly what heinous 'crime' Ptolemy here stands charged with Surely not mere manufacture of evidence in the face of modern canons of scientific propriety, or 'improving' existing data to give a better fit with his preferred structurings of the observable phenomenal reality? Let one look to the working papers of Kepler or Newton (Isaac) in the seventeenth century and see how innocent and amateurish Ptolemy's efforts to round out and confect empirical data were, even if all that is here alleged be true. And if his fabrications, such as they might have been, were indeed a gigantic confidence trick which deceived astronomers for the next millennium and a half and more, is it not that they were knowingly led to believe the myth of his supremacy? Granted that Ptolemy's model of Mercury's orbit makes such a poor fit with what of it can be glimpsed by the naked eye even of an Earth-bound observer, why did the highly competent Arabic astronomers al-Shirazi and his pupil al-Shatir content themselves with so minimal a reshaping of it, one all but exactly preserving Ptolemy's lines of sighting? Yet more astonishing, how was it that Copernicus should in Book 5 of his *De Revolutionibus,* that clarion call to heliocentric 'revolution' as it is so often mistakenly made out to be, timidly settle for parroting al-Shatir's version (as who will now gainsay) of Ptolemy's theory of that planet's motion, one in which the Sun is as ever in motion round the Earth (and cannot be made to stand still in any version of it)? The history of the impact of Ptolemy's *Syntaxis* upon succeeding centuries is a deal more subtle and convoluted than any simple 'Newtonian' view of it as poor theory shabbily propped up by invented observational data, and the whole fraudulently presented to posterity as well-founded celestial science in classical hypothetico-deductive mould can more than start to explain.

If one suspends disbelief at certain passages in it, Newton's book makes an excellent read, but it is only the beginning of a serious estimate of Ptolemy's achievement, frauds and all. ☐

D. T. Whiteside is Reader in the History of Mathematics at the University of Cambridge, UK.

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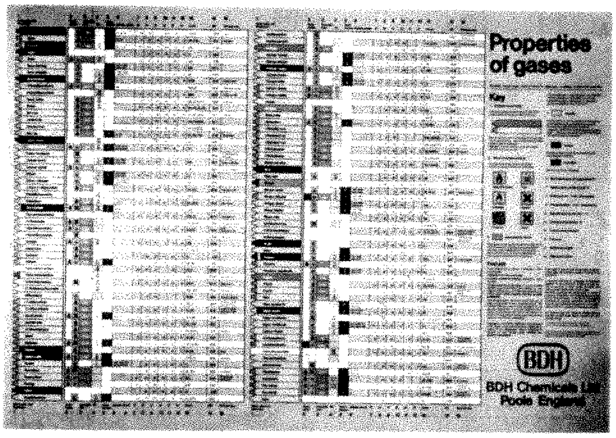

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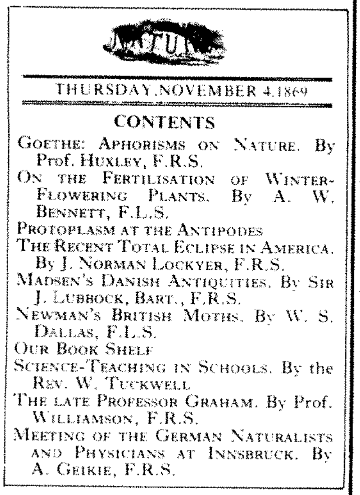

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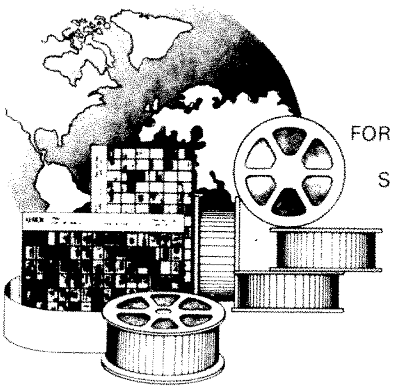

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articles

Hydration of obsidian

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The hydration of obsidian is studied by measuring hydrogen concentration profiles of natural samples hydrated at 90°C, using the ^{15}N resonance nuclear reaction method. The results of this study are well reproduced by the interdiffusion model of Doremus. The importance of the non-amorphous phase of obsidian is discussed. It is pointed out that the precision methods of measuring hydration profiles now available make it practical to measure hydration rates at room temperature experimentally.

THE study of the reaction between water and obsidian is important to many fields ranging from archaeology, through geochemistry and geophysics, to the technology of radioactive reactor waste consolidation and isolation. Here we investigate the process by which water hydrates a natural obsidian where the hydration is carried out in controlled laboratory conditions. The principal aim is to deal with aspects of the hydration mechanism as it relates to the archaeological technique of dating obsidian artefacts (arrowheads, knife blades, scrapers) based on the extent of hydration on the surface of the object, but the techniques used and conclusions drawn are quite general and should apply to the hydration of many types of glasses.

Understanding the details of the hydration mechanisms is especially important in archaeology to make the best quantitative date assignments based on the hydration[1–3]. Ideally, the archaeologist would like to be able to measure the extent of hydration on a given artefact and to have a method with which to deduce its rate of hydration. With these data he can assign a date. There are now several methods of varying complexity and accuracy for measuring the extent of hydration, and more methods are likely to be developed[4,5]. To be able to deduce the hydration rate, we clearly need to begin with an understanding of the hydration mechanism, and this is the aim of this article. Our general conclusion is that the hydration involves the interdiffusion of hydronium ions (H_3O^+) with mobile alkali ions in the glassy phase of the obsidian. The realisation that natural obsidians can have substantial non-amorphous phases is important for understanding some of the results presented here and elsewhere. Although the aim of the present work is to understand the hydration mechanism, it became clear, from measurements made, that the precision methods now available for measuring hydration profiles make it possible to measure hydration rates directly at room temperature.

Experimental

The obsidian samples used in this study were obtained by fracturing a large piece of 'mahogany' obsidian from the western US. The elemental composition of this obsidian is given in Table 1. The fracturing process produced many thin (few mm) pieces of obsidian with large flat fracture surfaces. This method of surface preparation was chosen both because it duplicates that used to make the archaeological artefacts commonly dated by hydration methods and because our experience with other glasses indicates that other techniques, such as sawing, can affect the apparent hydration rate of the glass. Although no detailed studies are yet available, the sawing may create a network of microcracks which can enhance the initial hydration.

Our experimental procedure is modelled on one developed in a detailed study of the hydration of a commercial soda-lime glass[6]. Our obsidian samples were hydrated for up to 348 h in a bath of temperature-regulated distilled water held at 90°C. The resulting hydration on the surface of the obsidian was profiled quantiatively using the ^{15}N hydrogen profiling method[7]. This uses a narrow isolated resonance in the $^{15}N + {}^1H \rightarrow {}^{12}C + {}^4He + \gamma$-ray reaction as a probe for hydrogen and measures the hydrogen concentration in any solid against depth into that solid. This technique has been previously used to measure hydration profiles in soda-lime glass and is fundamentally similar to that used by Lee *et al.*[8] who were the first to use a nuclear reaction method to profile the surface of hydrated obsidian.

The variable-energy ^{15}N beam from the Yale MP Tandem was used. The experimental arrangement had been calibrated previously, allowing the γ radiation yield curve to be converted to a plot of absolute hydrogen concentration against depth using an energy loss of ^{15}N of 2.0 MeV μm^{-1}. Typical beam intensity was 2×10^{-9} A with a beam spot of 10 mm^2. We were concerned that the ^{15}N beam might disturb the hydrogen distribution during the analysis and to check this, some of our measurements were repeated after completing the profile. These repeat measurements showed no change in the hydrogen distribution. Detailed description of the experimental system and a comparison between this method and other analytical techniques for measuring hydrogen profiles are given elsewhere[7,4].

Results

The measured hydrogen profiles for our hydrated obsidians are summarised in Figs 1 and 2. Because the rate of hydration is expected to be limited by diffusion, we assumed that the thickness of the hydration profile would increase proportionally to the square root of the hydration time. This is shown in Fig 1 where the measured depth into the sample at which the hydrogen concentration has decreased to half the value near the surface is plotted against the square root of the hydration time: the expected $t^{1/2}$ dependence is obtained.

Clearly, the hydration is proportional to $t^{1/2}$, and so to discuss the shape of the hydration profile, it is convenient to combine all the data by removing this explicit time dependence—that is, to plot hydrogen concentration against depth divided by the square root of the hydration time ($x/t^{1/2}$). This is shown in Fig 2. The typical error bars shown on a few of the points reflect only statistical uncertainties in the data. By thus combining all these data, the statistical uncertainty associated with individual data points as well as any local variations in the composition of the

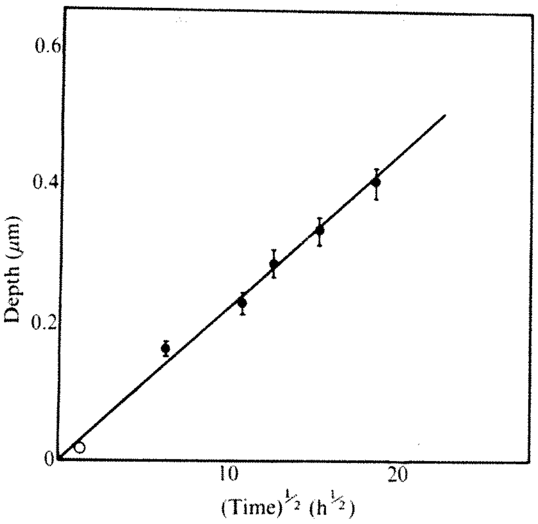

Fig. 1 The penetration depth as function of the square root of the hydration time. The penetration depth is defined as the depth to half-maximum hydrogen concentration. The error bars reflect the statistical uncertainties only. The open circle shows the penetration in an unhydrated sample exposed to air at room temperature for two months. The hydration time has been converted to the equivalent time at 90 °C by an extrapolation according to the Arrhenius equation assuming an activation energy of 20 kcal mol^{-1}.

obsidian become less important as we consider only the average profile.

The hydration profile shown in Fig. 2 has the shape expected from other experimental measurements[8,5] and for the Doremus interdiffusion model for the hydration of glasses[9]. In fact, the solid lines in Figs 1 and 2 result from a calculation based on this model. It is reassuring that the hydrogen content of the unhydrated obsidian (0.3×10^{21}H atoms cm^{-3}) deduced from the ^{15}N profile measurements agrees with the results of the commercial analysis of the obsidian's composition as given in Table 1.

Hydration mechanism

There has long been interest in the mechanism for the hydration of alkali glasses—such as obsidian—and there are several hypotheses on the important hydration mechanisms. We will compare our results with the Doremus model[9], in which the hydration is the result of ionic interdiffusion between the alkali ions in the glass with hydronium ions formed at the surface. Doremus compared the shape of the hydration profile predicted by his model with that measured by Lee *et al.*[8] on the surface of an archaeological artefact. Our more complete results presented here agree entirely with this first single comparison.

A second hydration mechanism which has been commonly mentioned is that of diffusion of molecular water into the glass matrix. We are not aware of reliable experimental evidence favouring this above the ionic interdiffusion model (although molecular water may diffuse into the ion-exchanged hydrated glass). One strong piece of evidence favouring the interdiffusion model is the depletion of alkali ions observed in hydrated glasses, including obsidians. This is observed both in leaching experiments where the amount of ions which diffuse out of the glass is measured, and in direct measurements of the profiles of the alkali elements in the hydrated glass[5,6]. A second general piece of evidence favouring the interdiffusion model is that the glasses which hydrate are ionic conductors whereas glasses without mobile ions do not generally hydrate (such as SiO_2).

The Doremus model[9] is based on the fact that in a glass such as obsidian, the only mobile charge carriers are monovalent alkali ions such as Na^+ and K^+. These positive ions are very mobile and would diffuse out of the glass were it not for the electrostatic attraction between them and the immobile anions in the glass. However, if some other positive ion such as hydronium (H_3O^+) is available to substitute for these alkali ions, the mobile alkali ions and the hydronium ions can interdiffuse, resulting in hydration of the glass. Hydronium ions are available from water on the surface of the glass reacting with Na in the glass: $2H_2O+Na^+ \text{(glass)} \rightarrow H_3O^+ \text{(glass)} + NaOH$ and through a similar reaction with K^+. We discuss H_3O^+ rather than H^+ or some other ion because results with other glasses indicate that about three hydrogen atoms replace every alkali atom during hydration[6]. The interdiffusion model is convenient because it can be transformed to an equivalent simple diffusion problem with a concentration-dependent diffusion coefficient $\tilde{D} = D_H D_A/(C_H D_H + C_A D_A)$ for which standard methods of solution exist where D_H and D_A are the diffusion coefficients of hydronium and the alkali ion, and C_H and C_A are their respective concentrations. Hence if one has a glass with a mobile alkali ion (such as Na^+) interdiffusing with hydronium, one can immediately predict the shape of the hydration profile and the rate of hydration given values of the diffusion coefficients of the Na^+ and H_3O^+. In the results presented here, the interdiffusion problem was solved numerically. In principle, when considering the profile shape one also has to consider the etching of the surface of the hydrated obsidian. However, the fact that the data fall in a straight line in Fig. 1 and that obsidians are known to hydrate to thicknesses of many microns implies the etching is very slow, so we have ignored it.

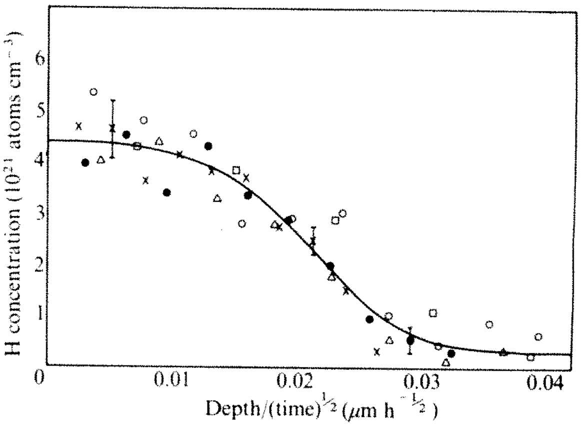

Fig. 2 The measured hydrogen profiles of the hydrated samples are here shown by using a reduced depth scale, which is the actual depth divided by the square root of the hydration time. The solid curve is the prediction of the interdiffusion model with $D_H = 1.4\times10^{-16}$ cm^2 s^{-1} and $D_A = 1.4\times10^{-14}$ cm^2 s^{-1}. The calculated profile has been normalised to the data in terms of the amount of hydrogen in the fully hydrated glass at the surface (see text). □, 40 h; △, 116 h; ○, 158 h; ●, 234 h; ×, 348 h.

In the present case, the glass contains both Na^+ and K^+ (see Table I), and both ions are expected to interdiffuse with hydronium. However, it turns out that as long as $D_A \gg D_H$ the hydrogen profile predicted by the interdiffusion model is not very sensitive to the precise value of the diffusion coefficient for the alkali ion (D_A). Increasing D_A by several orders of magnitude has only a small effect on the shape of the hydrogen profile and the rate of hydration. The underlying reason for this insensitivity to D_A is that as long as $D_A \gg D_H$ the rate-controlling step in the hydration process is the diffusion of hydronium into the glass.

Hence in a glass, such as the obsidian considered here, one expects the total measured hydrogen profile to represent the sum of two components (one for K and one for Na), but, as both profiles are similar, the sum will not be easily distinguished experimentally from that resulting from a single ion. Hence for simplicity we consider model calculations with only a single alkali ion, but the solution may be interpreted as the sum of two.

Figure 2 (solid line) shows the prediction of the interdiffusion model with $D_A = 1.4\times10^{-14}$ cm^2 s^{-1} and $D_H = 1.4\times10^{-16}$ cm^2

s^{-1}. As is evident, this prediction is in excellent agreement with the data. The shape of the predicted profile depends only on the ratio D_A/D_H, whereas the rate (shown in Fig. 1) is only weakly dependent on the ratio and proportional to D_H.

Because there are no measurements of D_A and D_H available, they were treated as free parameters to obtain the agreement between model predictions for the rate of hydration (Fig. 1) and the shape of the profile (Fig. 2). The diffusion coefficient for hydronium, thus obtained, is similar to that needed to understand the hydration of soda-lime glass and is consistent with other reported measurements[6]. The corresponding diffusion coefficient for the alkali ion is one order of magnitude smaller than that needed to understand the hydration of soda-lime glass[6]. However, the present obsidian has much less alkali than the soda-lime glass, which has 15.9% Na_2O, and the D_{Na} decreases rapidly with the concentration of Na^+ ions in the glass. Also, some of the alkali ions are K^+, which are less mobile than the Na^+, and hence D_A should be somewhat smaller than D_{Na}. Extrapolating the data for soda-lime glass[11] indicates that qualitatively we should expect D_A to be an order of magnitude smaller in this obsidian than in the soda-lime glass of ref. 6, which again agrees with our experimental results. There are also measurements of D_{Na} in obsidian in the temperature range 350–850 °C. Extrapolating these results to the 90 °C of the present hydration implies D_{Na} to be of the order of 5×10^{-14} with an uncertainty resulting from the extrapolation of at least an order of magnitude. Hence we see that the interdiffusion model predicts both the shape and absolute hydration rate with values of D_A and D_H (the only parameters in the theory) consistent with the available measurements of these diffusion coefficients.

One point, not discussed above, is important for reconciling the measurements reported here and their interpretation within the interdiffusion model with the results of others. The hydrogen profile shown in Fig. 2 has been arbitrarily normalised to the data in terms of the amount of hydrogen in the fully hydrated glass at the surface. If the hydrogen-bearing ion which replaces the alkali ions in the glass is hydronium, and if all the alkali ions are allowed to participate in the interdiffusion, then we expect the hydrogen content of the hydrated glass to correspond to a 3-atom hydrogen replacement for each alkali ion, as has been observed in manmade glasses (W.A.L. *et al.* unpublished and ref. 6). The results shown in Fig. 2 indicate only about half this amount of hydrogen. Measurements of the profiles of the alkali ion concentration in hydrated obsidian[5] also indicate that only about half of these ions are removed during the hydration, in contrast to the results in soda-lime glass where the sodium is completely removed.

The reason the alkali ions are only partially depleted in hydrated obsidian is not completely clear. One possibility is that some of the alkali ions are immobilised and, hence, cannot take part in the ion exchange. This immobilisation may result because in addition to the glassy phase, obsidians typically contain large amounts of crystal inclusions. A recent survey by Ericson *et al.* using optical methods indicated that ~10% of obsidians were crystal inclusions[13]. In addition to the crystal inclusions which can be seen microscopically, X-ray studies of purely 'glassy' obsidian show weakly developed lines which correspond to the minerals to which the obsidian would have crystallised had it cooled more slowly[14]. These X-ray studies indicate that obsidians can have small structures which, although not optically visible, may behave more like crystal inclusions than amorphous glass.

A second possibility is that the two alkali ions present (Na^+ and K^+) may interact immobilising some of the ions. This may be related to the dramatic decrease in ionic conductivity observed in mixed alkali glasses over those made with only one alkali element[10].

Table I Chemical analysis of obsidian

Chemical component	Weight %
SiO_2	75.85
Al_2O_3	12.81
Fe_2O_3	0.85
FeO	0.57
MnO	0.05
TiO_2	0.25
CaO	0.48
MgO	0.16
Na_2O	3.74
K_2O	4.20
H_2O^+	0.15
H_2O^-	0.008
P_2O_5	0.03
CO_2	0.05
Total	99.148

The analysis was carried out by York Research Corporation, Stamford, Connecticut, using atomic absorption spectroscopy and other methods.

The presence of a crystal component in obsidian has several implications. The crystal material is expected, if it hydrates at all, to hydrate many orders of magnitude more slowly than the glass. Feldspars contain proportionally much more of the Na and K than the bulk composition and, consequently, their existence substantially depletes the alkali component of the glass phase. A 10% component of potassium or sodium feldspar incorporates ~20% of the total alkali. In the present case, it is difficult to estimate quantitatively how much of the sodium and potassium given in the bulk composition (Table I) exists in the glassy phase and should then exchange with hydrogen in the hydration process: if 10% of the obsidian is crystallised in potassium or sodium feldspars[13] and if roughly an equal amount of alkali is immobilised in embryonic crystals, then the observed hydrogen content is consistent with each alkali ion being replaced with one H_3O^+ ion.

The suggestion that a fraction of the alkali ions in obsidian is immobilised is consistent not only with this interpretation of our present data but also with the recently reported[5] partial depletion of alkali ions in hydrated obsidian. It also provides a natural explanation for the fact that no correlation was found between alkali content and hydration rate for a variety of obsidians[1], whereas it is well known that for synthetic glasses the alkali content is key to the hydration[10]. If an unknown fraction of the alkali is immobilised in crystalline structures, the bulk alkali content may not reflect the true alkali content in the glass phase of the obsidian. On the other hand, the interdiffusion model predicts an increase in hydration rate with alkali content because of the known increase in alkali diffusion rate with increased alkali content. This should also be realised as a correlation between the maximum hydrogen content in fully hydrated obsidian with hydration rate. Such a correlation has already been reported in the hydrogen profile measurements of Lee *et al.*[8] The importance of the non-amorphous phase of obsidian in explaining the lack of correlations between bulk composition and density has already been reported[13].

Direct measurement of hydration rate at room temperature

The ^{15}N hydrogen profiling method can quantitatively measure hydrogen profiles as thin as a few hundred Angstroms. If the thickness of the hydrated surface layer (X) varies with time as $X^2 = KT$, archaeological evidence shows K varies from <1 to 20 μm^2 per 1,000 yr (ref. 2). Assuming, for example, $K = 10\ \mu m^2$ per 1,000 yr means that, if a piece of obsidian is fractured and the resulting fresh fracture surface is exposed to the ambient environment for one year, the resulting hydration layer will be 1,000 Å thick. This is easily measurable by the ^{15}N hydrogen profiling method. By such a procedure, it should be possible to determine the hydration rate for individual artefacts.

For example, the open data point in Fig. 1 is the hydration thickness (200 Å) of a sample of obsidian which was not exposed to the 90 °C water bath but which, instead, was in an air environment for two months before being profiled. The hydration rate deduced corresponds to $K = 2.4\ \mu\text{m}^2$ per 1,000 yr, which agrees with that predicted from the archaeological results.

A practical use of such measurements might be to fracture the artefact to be dated and return it to the environment in which is was found for one year; by measuring the hydration profile on the 'one-year-old' surface, a hydration rate is determined which is automatically averaged over annual temperature variations. By then measuring the hydration profile on an original surface, the age of the artefact could be deduced.

Conclusion

We see that the interdiffusion model predicts the shape of the hydration profile and the rate of hydration in good agreement with our data. The values of the two free parameters in this model (D_A and D_H) which are needed for agreement are, as well as can be presently determined, consistent with other measurements. Finally, we emphasise that the experimental methods described here can be useful in characterising the hydration rates of other glasses, whether the glass is a single archaeological artefact[2,15], a scientific or commercial glass, or a glass designed to isolate radioactive reactor wastes.

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Oceanic plate motions driven by lithospheric thickening and subducted slabs

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The thickening of the cooling oceanic lithosphere results in a significant driving force that is distributed over the area of the plates. Simple flow models show that this force may be more important in driving oceanic plates than that force from sinking lithospheric slabs at subduction zones.

IN the past decade, the theory of plate tectonics has been quite successful in explaining many geological and geophysical observations. Nevertheless, plate tectonics remains primarily a kinematic theory. Although some form of thermal convection must be responsible for the plate motions[1], the details of the driving mechanism and flow in the mantle accompanying the plate motions are still uncertain. The lithosphere, the mechanically strong thermal boundary layer which makes up the plates, probably has an important influence on mantle flow: the largest temperature gradients in the Earth occur across the lithosphere. As convection is driven by the body forces caused by differences in temperature, the plates might be expected to play an important part in governing convection in the Earth. Thus, to understand the dynamics of mantle convection, it is important to isolate and understand the effects resulting from the plates themselves.

Because the plates are denser than the mantle below, they are gravitationally unstable and ultimately sink into the mantle at subduction zones. The sinking of the dense subducted slabs into the mantle provides a potentially important driving mechanism for plate motions which has been considered in several investigations of the forces driving the plates[1–4].

Important density contrasts are not, however, limited to the sinking slabs. As the oceanic lithosphere moves away from the ridge where it is created, it becomes thicker because cooling penetrates deeper into the mantle. This thickening of the dense lithosphere leads to horizontal density differences across distances of the order of the plate dimensions. Simple flow models presented here show that these density contrasts are sufficient to drive plate motions at rates of centimetres per year. In fact, the body forces from the thickening of the lithosphere seem to be even more important in driving plates than the body forces associated with the subducted slabs.

Model of the lithosphere

The thermal evolution of the oceanic lithosphere is fairly well understood. Many of the properties of the oceanic lithosphere can be explained by simple models based on cooling the mantle from above[1,5–8]. The essential features of these models are illustrated by a model of a cooling halfspace[6,7]. In this model, shown in Fig. 1, mantle material at temperature T_0 rises in the vicinity of an oceanic ridge and moves horizontally away from the ridge at a velocity u which is constant and independent of depth. At distances not too close to the ridge, the temperature at depth ζ is given in terms of an error function:

$$T(z) = T_0 \operatorname{erf}\left(\zeta/2(\kappa t)^{1/2}\right) = T_0 \operatorname{erf}\left(\zeta/2(\kappa x/u)^{1/2}\right)$$

where κ is the thermal diffusivity, t is the time since the material was at the ridge, and x is the horizontal distance from the ridge. The second equality results from the relationship between time and distance from the ridge, assuming constant spreading velocity u.

If the base of the thermal boundary layer constituting the lithosphere is defined by some critical temperature T_l, the thickness of the lithosphere d is given by

$$d = 2(\kappa x/u)^{1/2} \operatorname{erf}^{-1}(T_l/T_0)$$

It is a property of the error function solution that the average temperature, and therefore the average density, of the lithosphere is independent of distance from the ridge. Thus the lithosphere can be thought of as a cool, dense, mechanically strong layer of constant mean density which grows thicker away from the ridge as the material formerly below the lithosphere cools and becomes part of it.

As the dense lithosphere thickens by accretion, it subsides into the less dense mantle beneath, resulting in an increase in ocean depth away from the spreading ridge. This sinking is

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inferred to lead to an essentially complete isostatic compensation of the thickening lithosphere because the free air gravity anomalies observed over oceanic ridges and basins are small[9]. Isostasy requires that the elevation of the seafloor, η_t, and the elevation of the base of the lithosphere, η_b, both referred to their final depths just before subduction, are related by

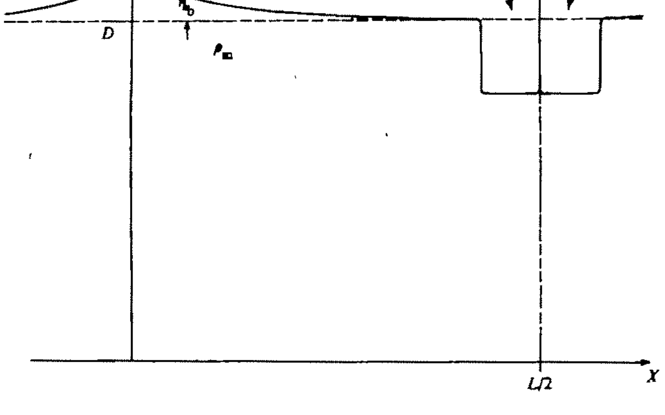
$$\eta_b = ((\rho_l - \rho_w)/(\rho_l - \rho_m))\eta_t$$

where ρ_l, ρ_w, and ρ_m are the densities of the lithosphere, seawater, and the mantle, respectively. The boundary layer theory predicts that the increase in depth of the ocean owing to the isostatically compensated thermal contraction of the surface layer should depend on the square root of age or distance from the ridge crest, in accord with observation to plate ages of at least 80 Myr (ref. 8).

Eventually, the dense plate plunges into the mantle at a subduction zone. As it sinks it may pull the rest of the plate with it, as suggested by Elsasser[10]. Simple dynamic models of mantle flow[4] and phenomenological studies of forces on plates[2,3] have indicated that the body forces of the sinking slab may provide an important part of the forces driving the plates, although detailed models involving the transmission of the force from the sinking slab to the horizontal plate have not yet been analysed.

The thickening of the lithosphere itself is another source of density contrast that can cause flow and plate motion. Figure 1 shows that the dense lithosphere is separated from the less dense mantle below by a sloping interface. The situation is analogous to a dense fluid overlying a less dense fluid in a tank. Clearly, if the interface between the two fluids slopes, the configuration is not in equilibrium, and the dense fluid will begin to sink at its thick end while the less dense fluid will rise at the other end where the dense layer is thinnest. Similarly, the thick lithosphere far from the ridge will sink, and the less dense mantle will rise under the ridge. There will be an accompanying surface flow from ridge to trench, corresponding to plate motion, with a return flow at depth to complete the mass circuit. As the mantle material rises continuously under the ridge, it cools and accretes onto the dense lithosphere. The lithosphere is reheated and resorbed as it sinks into the mantle; this would cause steady state thermal convection driven by body forces associated with the thickening of the lithosphere at the surface if accretion and resorption occurred at the appropriate rate.

Fig. 1 Schematic representation of the geometry assumed in the flow models. The elevation of the sea floor, η_t, and the elevation of the base of the lithosphere, η_b, are related because the lithosphere is in isostatic equilibrium. Because the density of the mantle, ρ_m, is less than the density of the lithosphere, ρ_l, horizontal density contrasts due to the thickening lithosphere and subducted slab drive the plate towards the trench. The core-mantle boundary is at $z = 0$.



The velocity of this plate-driven flow depends on the extent of the horizontal density differences. If the top and bottom of the lithosphere were flat, there would be no horizontal density differences, so there would be no motion. One measure of the magnitude of the driving force is the horizontal mass difference, the volume integral of the horizontal density contrast. If isostatic compensation is assumed, the horizontal mass difference accompanying the change in depth of the base of the lithosphere is equal in magnitude to the horizontal mass difference accompanying the change in elevation of the sea floor. The total horizontal mass difference is 9.2×10^{12} kg m^{-1} for a ridge whose elevation decreases as the square root of the distance, from 3 km at the ridge crest to 0 km, 6,000 km away assuming a density difference of 2.3 Mg m^{-3} between the lithosphere and seawater.

The total horizontal mass difference inherent in the subducted slab is not as well constrained as that resulting from the thickening of the cooling plate because the thermal evolution of a subducted slab depends on complicating factors such as viscous dissipation, phase changes, and the variation of the ambient temperature of the mantle with depth[11]. In a model of the evolution of a 120-km thick slab calculated by Schubert *et al.*[12], including the effects of frictional heating and phase transitions, the horizontal mass difference is 4.5×10^{12} kg m^{-1}, about a third of which results from elevation of the phase boundary between the olivine and spinel phases at a depth of 350 km. This model predicts a much larger horizontal mass difference than other slab models[1,11] but this mass difference is still a factor of 2 smaller than that calculated for the thickening of the cooling surface plate. Thus the body force associated with the cooling plate may be more important in driving plate motions and mantle flow than the body forces associated with the sinking slabs. Of course, the flow in the mantle owing to a distribution of body forces depends on the position as well as the magnitude of the body forces, so it is necessary to compute flow models before evaluating the relative importance of these two body forces resulting from the thermal evolution of the lithosphere.

Viscous flow models

To facilitate comparison of the relative magnitudes of the two driving mechanisms, the flow driven by the thickening lithosphere alone is computed for comparison with the flow driven solely by the sinking slab. The rheology of the mantle is taken as newtonian viscous and the fluid is incompressible, which is consistent with the commonly used Boussinesq approximation Neither assumption is strictly justified for flow in the Earth, but this is not expected to invalidate the qualitative conclusions to be drawn from the models. Plate geometries and the distribution of body forces are assumed to be in steady state, so there is no time dependence in the models; streamlines and particle paths coincide.

The Stokes equations for a fluid in a two-dimensional Cartesian frame are solved by decomposing horizontal variations of the flow variables into Fourier series and solving for their depth dependences for each term in the series Where volumetric density variations exist, they are expressed as equivalent surface density distributions on planes spaced closely enough that the computed flow pattern is not affected appreciably by this approximation.

The shear stress is zero at the top and bottom of the model, which correspond to the ocean floor and core-mantle boundary. According to standard practice[6,19], no vertical displacement of these boundaries is permitted. The vertical displacement which would result if a boundary were permitted to deform is, to first order, the topography which could be supported by the normal stress at the undeformed boundary. As this topography is negligible compared to the depth of the model, the flow geometry is not appreciably affected by this approximation. The boundary conditions at the sides are also free slip, equivalent to the periodic array of plates shown in Fig. 1. The width of each cell is 5,570 km. The models shown below have a viscosity of 10^{21} N s m^{-2} throughout, the average mantle viscosity determined in studies of postglacial rebound[13].

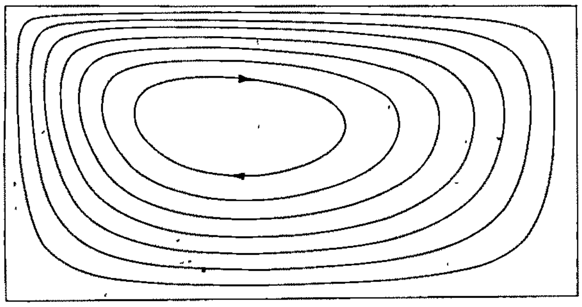


Fig. 2 Streamlines for flow driven only by the thickening lithosphere. The maximum magnitude of the stream function is 8.2×10^7 cm^2 yr^{-1} and the contours are equally spaced. The average surface velocity is 1 cm yr^{-1}. The maximum velocity is 1.6 cm yr^{-1} and occurs at the surface. The viscosity is 10^{21} N s m^{-2} throughout.

The streamlines for flow driven by the thickening lithosphere are shown in Fig. 2. The horizontal density contrast from the thickening of the plate is included at a depth of 64 km. The density contrast is that needed to compensate for a seafloor elevation decreasing as the square root of the distance from the ridge from a maximum elevation of 3.0 km, the topography appropriate for a spreading rate of 7 cm yr^{-1}. A density contrast between the lithosphere and seawater of 2.3 Mg m^{-3} is assumed.

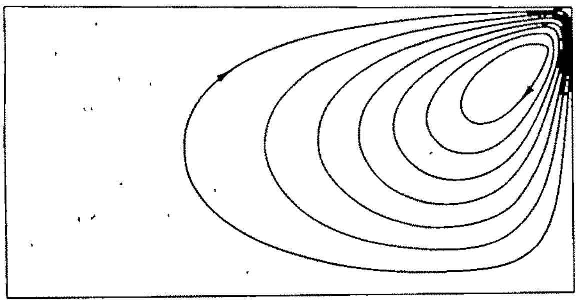
The average velocity at the surface is 1 cm yr^{-1}. Near the surface, the streamlines are essentially parallel, indicating that the flow driven by the thickening lithosphere causes little deformation of the surface. Calculated shear stresses are of the order of 0.5 MPa.

Details of the flow depend on the viscosity structure. Increasing the viscosity in the lithospheric layer by a factor of 10 decreases the average surface velocity by a factor of 2. The average surface velocity increases to 16 cm yr^{-1} if a 64-km thick low viscosity channel of 10^{18} N s m^{-2}, similar to that proposed to lie under oceans by Artyushkov[14], is included at the base of the lithosphere. One could easily match the surface velocity of 7 cm yr^{-1} with the density distribution driving the flow in this model by a suitable selection of parameters and hence the model of the plate-driven flow can be made entirely self-consistent.

The streamlines for flow driven by the sinking of a subducted slab are shown in Fig. 3. The density contrast in the slab decreases linearly with depth from 0.06 Mg m^{-3} at the surface to zero at a depth of 670 km. This decrease in density with depth, which might result from heating the slab as it sinks, was chosen so that the calculated stresses would roughly match the seismic observation that slabs are in down-dip compression below a depth of 300 km (ref. 15).

The average velocity at the surface is 1 cm yr^{-1}, although the velocity reaches 8 cm yr^{-1} in the region of the sinking slab, where the flow is concentrated. The converging streamlines along the surface indicate that the near-surface layers experience extensional stress.

Fig. 3 Streamlines for flow driven by a sinking slab. The density contrast in the slab varies linearly from 0.06 Mg m^{-3} at the surface to 0 Mg m^{-3} at a depth of 670 km. The maximum amplitude of the stream function is 14.8×10^7 cm^2 yr^{-1} and the contours are equally spaced. The average surface velocity is 1 cm yr^{-1}, although vertical velocities in the subducted slab reach 8 cm yr^{-1}. The viscosity is 10^{21} N s m^{-2} throughout.



As before, varying the viscosity structure changes the flow somewhat. Increasing the viscosity of the lithosphere by an order of magnitude decreases the average surface velocity by a factor of 2. Alternatively, including the 64 km thick low viscosity channel of 10^{18} Ns m^{-2} leads to an average surface velocity of 6 cm yr^{-1}, compared with 16 cm yr^{-1} for the same viscosity distribution with the flow driven by the thickening lithosphere.

Discussion

For the constant viscosity models, the average surface flow driven by the thickening lithosphere is as large as that driven by the sinking slab. A low viscosity channel increases the surface flow for the thickening lithosphere model more than for the slab model. Also, the 670-km slab in the model is longer than the seismicly determined length of most subducted slabs[15]. Therefore, on a worldwide basis, the surface flow driven by the thickening oceanic plates might well be larger than the surface flow driven by subducted slabs.

For viscous flow driven by body forces, the scale of the flow depends on the distribution of those forces. The density differences associated with thickening oceanic plates are spread out over distances equal to the dimensions of the plates, while the body forces associated with subducted slabs are more localised. The flow driven by the sinking slabs is concentrated in the region of subduction, where it may be important in the local deformation associated with bending and underthrusting of the lithosphere. In contrast, the flow driven by plate thickening is more global in scale, in accord with plate motions that are coherent over large distances.

The predicted dependence of ocean depth on the square root of age is not valid beyond about 80 Myr, which corresponds to lithospheric thickness on the order of 130 km, after which the thickness may not increase appreciably[8]. Consequently the driving force from the thickening lithosphere is distributed primarily over that part of a plate which is <80 Myr old. For very slowly spreading plates, this force can probably be approximated as a boundary force acting on the ridge itself[1-4,14,16]. Nevertheless, as Hales[17] and Lister[18] have pointed out, for spreading rates typical of oceanic plates, this driving force will be distributed over substantial areas of the plates.

The distinction between an edge force and a distributed force has important dynamic implications. The velocities of oceanic plates are observed to be relatively independent of area; the small Cocos Plate, the intermediate Nazca Plate, and the large Pacific Plate all move at about the same rate[2]. For plates spreading at equal rates, the driving force from the thickening lithosphere is greater for large plates than for small ones. Also, the body force in the subducted slab, which must depend to a large extent on the time a plate cooled at the surface before subducting, should be greater for a large, old plate than for a small, young one. For the plate velocities to be relatively independent of area, there must be a resisting force which increases as the area of a plate increases. Drag at the base of the lithosphere is likely to satisfy this criterion.

For drag at the base of the lithosphere to be an important resisting force, oceanic plates, on average, must be moving faster than the underlying mantle. Richter showed that the shear generated by a moving lithosphere should exert a strong organising influence on the flow in the mantle, resulting in a coherent large scale convection pattern dominated by the plate motions[19].

The nature of the force resulting from the thickening of the oceanic lithosphere suggests that oceanic plate velocities might be self-limiting. The slope at the base of the lithosphere is greater for slowly moving plates than for rapidly moving ones. Therefore, the driving force from the thickening of the plates is larger for slowly moving plates, all other factors being equal. If a plate were to move away from a ridge more rapidly, the ridge topography and accompanying topography at the base of the lithosphere would become flatter, providing less drive. Oceanic plates might all move at about the same speed[2] because their associated ridges are spreading at terminal velocity, rather than because their slabs are sinking at terminal velocity[2–4], as old slabs might be expected to have a larger terminal velocity than young ones.

Conclusions

The density contrast at the Earth's surface associated with the cooling and thickening of the oceanic lithosphere provides a significant driving force for plate motions. Simple numerical models of mantle flow show that this plate-driven flow may be more important in generating coherent surface motions than the sinking slab, although flow driven by the sinking slab is likely to be important locally in the region of subduction. Although these models do not consider how oceanic plate motions originate, they do show that the horizontal density differences resulting from the cooling of these plates are capable of sustaining this motion.

The driving force on a plate from the thickening oceanic lithosphere is distributed over the area of the plate. As oceanic plate velocities do not depend on plate area, there is likely to be a significant resisting drag proportional to area at the base of the lithosphere. Thus the plate motions should be important in determining the large scale flow in the mantle.

The magnitude of the plate-driven flow depends on the slope of the boundary between the dense lithosphere and the underlying mantle. As this slope decreases as spreading rate increases, plate-driven flow may be self-limiting, helping to account for the observation that oceanic plate velocities show little variation.

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Colicin K acts by forming voltage-dependent channels in phospholipid bilayer membranes

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The bactericidal action of colicins K, E1, Ia, and other functionally related colicins involves disruption of active transport and leakage of ions from the cell. We show that a single colicin K molecule can form a voltage-dependent, relatively nonselective, ion-permeable channel of a few picosiemens conductance in a planar phospholipid bilayer membrane. In a membrane containing many of these channels, the ratio of the number of conducting to nonconducting channels changes e-fold per 3.7 mV. We suggest that the physiological effects of colicin K and functionally related colicins result from their ability to form ion-permeable channels in the bacterial plasma membrane.

COLICINS, bactericidal proteins (MW 50,000–100,000) produced by *Escherichia coli*, are of three major functional types: E1, E2 and E3. (For reviews of the physiology of colicin action see refs 1 and 2.) The cellular targets of action of types E2 and E3 are DNA and ribosomal RNA, respectively. The precise target and mechanism of action of the E1 type are not known, but these colicins are thought to act by making ion-permeable channels in the bacterial plasma membrane because: (1) they discharge the 'energised state' of the plasma membrane[1–7] without inhibiting respiration[1,8,9] or inducing measurable proton flux[6,7,10,11], and (2), an early effect of their action is net efflux of potassium from the bacterium[10,12–17]. (As macroscopic electroneutrality requires that net potassium efflux be accompanied by either efflux of anions and/or influx of other cations, the colicin-induced permeability is apparently rather nonselective.) Channel formation by these colicins is also consistent with observed single hit killing[18–20], and a *p*H or voltage dependence of the induced channels would explain the protective action of uncouplers of oxidative phosphorylation (such as FCCP) and of other treatments that discharge the energised state of the plasma membrane[16,18,21].

We report here that colicins K, E1, and Ia (members of the E1 functional type) form voltage-dependent, relatively nonselective ion-permeable channels in planar phospholipid bilayer membranes. We believe that this is their primary action on cells and accounts for the observations cited above. We present here data obtained from colicin K; closely related effects are obtained with E1 and Ia.

Conductance is a function of voltage

Planar phospholipid bilayer membranes (area $\approx 0.01\ \text{mm}^2$) separating two salt solutions were formed at room temperature by the union of two monolayers[22] of crude soybean phospholipid (lecithin type II from Sigma)[23] from which neutral lipid was removed[24]. After addition of colicin K to the front (or *cis*) compartment to a concentration of from 0.2 to $200\ \text{ng ml}^{-1}$, known voltages were applied across the membrane, and the resulting current responses were measured[25]. The conductance (g) of the membrane in symmetric salt solutions is defined as current divided by voltage ($g = I/V$), where V is the potential of the rear (or *trans*) compartment; in the absence of colicin, g is about 10 pS.

Figure 1 shows the typical response of a membrane which separates symmetric salt solutions and has been treated with

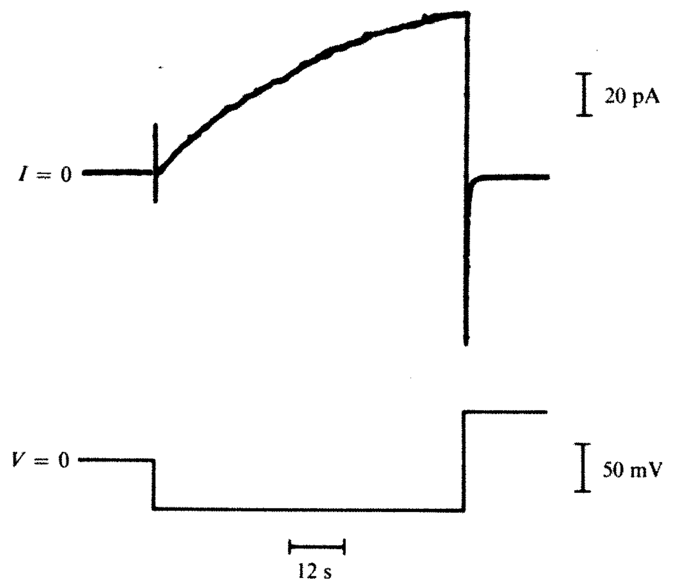

Fig. 1 Current response of colicin K-treated membrane to voltage. After formation of the membrane (in 100 mM KCl + 5 mM $CaCl_2$ + 1 μM EDTA + 5 mM 2[*N*-morpholino]ethane sulphonic acid, all adjusted to *p*H 6.1 with KOH), colicin K produced by *E. coli* was added to the front (or *cis*) compartment to a concentration of 100 ng ml^{-1}, and the record was obtained about 10 min later. The *cis* compartment was connected to virtual ground; *V* is the potential of the rear (or *trans*) compartment, analogous to the inside of the target bacterium. At the 'blip', a voltage of −50 mV was applied across the membrane. The current rises with approximately $1-\exp(-t/\tau)$ kinetics ($\tau \approx 25$ s) to a steady-state value of 80 pA, which corresponds to a conductance of 1,600 pS. At this point the potential was reversed to +50 mV. The instantaneous current is of the same magnitude but opposite sign as that at −50 mV, and decays rapidly to a very low value. The steady-state colicin-induced conductance at −50 mV is 400 times that observed at +50 mV. Stock solutions of colicin (~1 mg ml^{-1}) in 500 mM KCl + 50 mM potassium phosphate (*p*H 6.0) or in 300 mM NaCl + 50 mM Tris (*p*H 7.4) were stored in small vials at −70 °C. For a given day's experiments, the contents of a vial were thawed and, along with subsequent dilutions, stored on ice. Colicin K handled in this way was indefinitely stable (>6 months) with respect to its action on lipid bilayers; when left at room temperature for a day or two, however, it lost virtually all of its activity on bilayers.

colicin K on one side. When a negative voltage of sufficient magnitude (in this case −50 mv) is applied, current (and therefore conductance) rises with approximately $[1-\exp(t/\tau)]$ kinetics, with τ in this case about 25 s and the steady-state conductance about 1,600 pS. If the sign of the voltage is reversed after this rise, the current initially has the same magnitude (but, of course, the opposite sign), and then decays rapidly within a second to a low value little greater than that for an unmodified membrane. The steady-state conductance is extraordinarily voltage dependent (Fig. 2). Conductance is maximal at large negative membrane potentials and is virtually zero at potentials > -10 mv (Fig. 2*a*). τ Is also voltage dependent, decreasing as $|V-V_0|$ increases. (See below for definition of V_0.) Over long periods ($t \gg \tau$), there is a slow linear rise in the magnitude of the voltage-dependent conductance.

Single channel behaviour

The colicin-induced conductance described above is due to ion-permeable channels. At small colicin concentrations, discrete current 'jumps' are seen at constant voltage (Fig. 3). The conductances corresponding to these jumps are fairly uniform and, in 100 mM KCl, are about 1.5–3 pS for colicin K produced by *E. coli*. For colicin K produced by *Proteus mirabilis*, the jumps are about 10 pS; this surprising difference is discussed below. These unit conductance jumps are much too large for a shuttling ion carrier, and must therefore result from ion-permeable channels[26,27]; they are comparable in size to those of the sodium and potassium channels in nerve and the cholinergic channels at the neuromuscular junction[28]. The linear dependence of maximal steady-state conductance on colicin concentration (Fig. 4) implies that each voltage-dependent channel is formed by a single colicin K molecule.

The macroscopic voltage dependence (Fig. 2) is reflected in single channel behaviour (Fig. 3); that is, channels are almost always on at large negative voltages and almost always off at small negative or positive voltages. The data in Fig. 2*a* are replotted in Fig. 2*b* according to the Boltzmann relationship[25]. Over a wide voltage range the ratio of the number of open to closed channels changes *e*-fold per 3.7 mV. The potential at which half the channels are open (ratio = 1) defines the 'switching voltage' (V_0) of the channel, in this case −31 mV.

Similar results were obtained in membranes formed from whole *E. coli* phospholipids or from mixtures of purified bacterial phospholipids mimicking that composition. The number of channels obtained for a given concentration of colicin was 1–10% of the number obtained with membranes formed from crude soybean phospholipids.

Ion selectivity

Colicin K-induced channels are cation selective, but poorly so. A ratio of 10:1 for NaCl concentrations across the membrane (50 mM:500 mM) produces a diffusion potential of 33 mV (dilute NaCl side positive). This was established by determining the reversal potential either for the macroscopic current produced by many channels or for the individual current produced by a single channel (Fig. 5). This potential implies a selectivity of Na^+ to Cl^- of about 4:1. Essentially the same result is obtained with gradients of KCl, indicating little discrimination by the channel between K^+ and Na^+; the absence of a significant bi-ionic potential for KCl/NaCl confirms this. The channel also admits divalent cations; in a fivefold gradient of $CaCl_2$ (20 mM:100 mM) the diffusion potential is approximately 2.5 mV (dilute side negative), indicating a slight preference for Ca^{2+} over Cl^-.

The colicin molecule itself forms the channel

Addition of colicin K preparations to one side of planar phospholipid bilayer membranes induces the formation of strongly voltage-dependent, relatively nonselective channels. It is necessary to establish that these channels are not produced by a contaminant in the preparations, rather than by the colicin itself, because the actual number of channels induced in the membrane represents a small fraction of the number of molecules added to the aqueous phase. [This low efficiency is not surprising; it is generally seen with channel-forming molecules (such as gramicidin A) added to the aqueous phase, and probably results from slow kinetics of insertion into the membrane as well as nonspecific adsorption to other surfaces.]

We think it is very unlikely that a contaminant produces these channels. First, these channels are obtained from two separate preparations of colicin K, including a very pure sample from *P. mirabilis*. Second, we have found that colicins E1 and Ia, which act very much like colicin K on cells[1,2,17], also create voltage-dependent, ion-permeable channels in planar phospholipid bilayer membranes. (Whereas the behaviour of colicin Ia is qualitatively similar to that of colicin K, preliminary experiments indicate that the details of the voltage-dependent step(s) in colicin E1 action may be somewhat different.) In contrast, colicins E2 and E3, whose known targets are DNA and rRNA, respectively[1,2], are without effect on the bilayers—with the following caveat. At colicin E2 concentrations 1,000 times larger than those used in colicin K, E1 or Ia experiments, we obtained comparable channel activity. We suspect that this resulted from a small amount of Ia contamination of the E2 preparation; this is not unexpected, as the bacterial source of E2 also carried the Ia gene (T. Boon, personal communication). Furthermore, the single-channel conductance in 100 mM KCl

(~7 pS) and the kinetics of the 'E2 contaminant' were indistinguishable from those of Ia. Also, J. Konisky (personal communication) has found that E2 has a small amount of Ia-like activity which is neutralised by anti-Ia antibodies. The permeability effects observed with E2 on cells[29] may result from this same contamination. Third, two colicins K, produced by supposedly identical plasmids in *E. coli* and *P. mirabilis*[30,31], differ in their single channel conductances (see above). Two independent laboratories have since found that these two colicins differ in molecular weight as determined by SDS-polyacrylamide gel electrophoresis. Colicin K prepared from *E. coli* has a molecular weight of about 5,000 less than that of colicin K prepared from *P. mirabilis* (S. E. Luria and C. Kayalar; K. Jakes, personal communications). Thus, there is a correlated difference in artificial membrane behaviour and biochemistry.

These findings leave little doubt that the channel activity described in this article is produced by colicin K.

Channel activity explains cellular pathophysiology

We believe that the ability of colicin K to make channels in lipid bilayer membranes accounts for the salient features of its action on bacteria as summarised below. In considering this action, we do not address the question of how the colicin, through interaction with its receptor in the outer bacterial membrane, reaches the plasma membrane[32]. Our analysis depends on its somehow doing this. We expect that colicin K and functionally related colicins (such as E1 and Ia) should be effective on suitably prepared spheroplasts and vesicles of both sensitive and resistant strains. In fact, there are already reports of this[7,12,32–36]. The difficulties encountered in obtaining effects with these colicins on bacterial vesicles may be related to the low sensitivity to colicin action of membranes made from *E. coli* phospholipids (see above), and perhaps one function of the receptors in the outer bacterial membrane is to promote the penetration of these colicins into the plasma membrane.

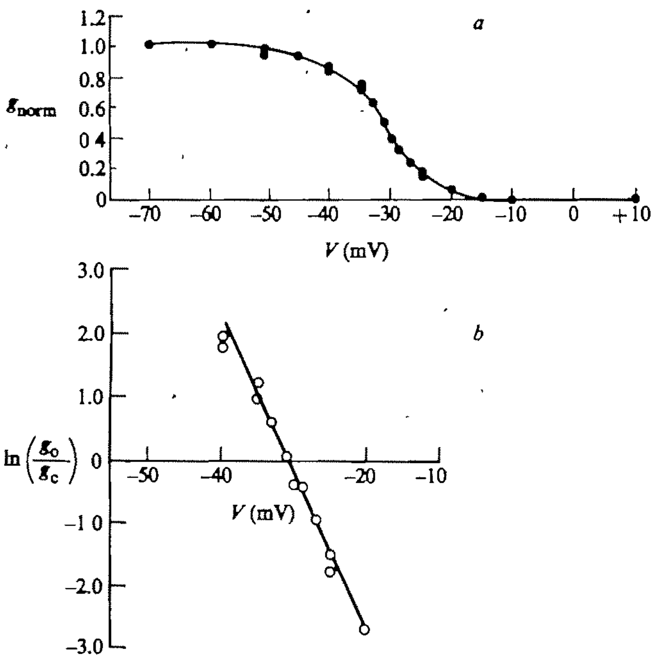

Fig. 2 Voltage dependence of the colicin K-induced conductance *a*, Normalised steady-state, colicin K-induced conductance (g_{norm}) plotted as a function of voltage. (Conductances normalised by setting the conductance at −60 mV equal to 1.) Colicin K (from *E. coli*) was present in the front (*cis*) compartment at 100 ng ml^{-1} Note that the voltage at which $g_{norm} = 1/2$, the 'switching voltage', is −31 mV, and that for voltages > -10 mV, $g_{norm} \sim 0$. The composition of the aqueous solutions are as for Fig. 1. *b*, Plot of ln g_0/g_c against voltage, where g_0 is the fraction of channels in the open (conducting) state, and g_c is the fraction of channels in the closed (nonconducting) state. This is a replot of the data from (*a*); see ref. 25 for details. Note that the points lie on a straight line, and therefore the data are consistent with the Boltzmann relationship:

$$g_0/g_c = \exp\left[-nq(V - V_0)/kT\right]$$

From the slope of the line we see that g_0/g_c (the ratio of the number of open to closed channels) changes *e*-fold for every 3.7 mV. Thus *n* in the above equation is 6.9, suggesting that approximately 7 'equivalent gating charges' are involved in switching a channel on and off. From the intercept of the line with the abscissa, we find that V_0, the switching voltage, is −31 mV. (In over 30 membranes, the value of *n* was consistently between 5 and 7; V_0 is usually around −30 mV, but occasionally values as high as −60 mV have been observed.)

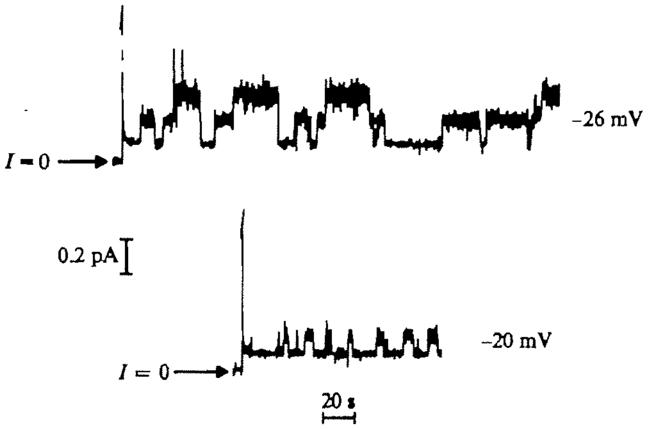

Fig. 3 Current (conductance) fluctuations due to the presence of two colicin K channels in the membrane The large transients mark the times when the voltage was switched from 0 mV to the designated values The conductance of a single channel in the 500 mM KCl solution is about 7.5 pS and is voltage independent. (The size of the current fluctuations is directly proportional to the applied voltage and hence is smaller at −20 mV than at −26 mV.) Note that at −20 mV ($V > V_0$) the channels tend to remain off. At −26 mV ($V \sim V_0$) there are times when both channels are on, one is on, and both are off—with a mean of about 1 channel on. For large negative voltages ($V \ll V_0$) both channels are on most of the time (data not shown). Colicin K was from *E. coli*. The membrane separates identical salt solutions consisting of 500 mM KCl + 5 mM $CaCl_2$ + 1 μM EDTA + 5 mM MES (all adjusted to *p*H 6.1 with KOH)

Ion flux and single hit killing

An early effect on cells of E1 type colicins is the promotion of ion flux[10,12–17]. At low colicin K multiplicity, the rate of potassium efflux from cells suspended in NaCl is directly proportional to colicin concentration; a single active molecule depletes a bacterium of 90% of its potassium in about 10 min[15]. Implicit in this net loss of potassium is an influx of cations (in this case Na^+) and/or an efflux of anions (perhaps Cl^-).

The properties of the colicin K channel observed on planar phospholipid bilayer membranes account for the *in vivo* flux data. The poor ion selectivity of the channel permits sodium and chloride, as well as potassium, movement. Furthermore, the magnitude of the channel conductance is consistent with the rates of potassium efflux observed by Wendt[15], who suspended cells in 20 mM NaCl. The internal potassium concentration of the cells is about 200 mM (ref. 37). Given the selectivity of the colicin channel, it is likely that in Wendt's experiments the membrane potential of the bacteria very shortly after the insertion of colicin channels was around −58 mV. In these conditions we calculate an initial rate of potassium efflux through the colicin K channel (from *Proteus*) of approximately 10^{-18} mol s^{-1}, in agreement with Wendt's[15] data, which translate into an initial rate of potassium efflux of around 5×10^{-19} mol s^{-1} per killing unit. The linear dependence of conductance on colicin concentration (Fig. 4) is also consistent with Wendt's results.

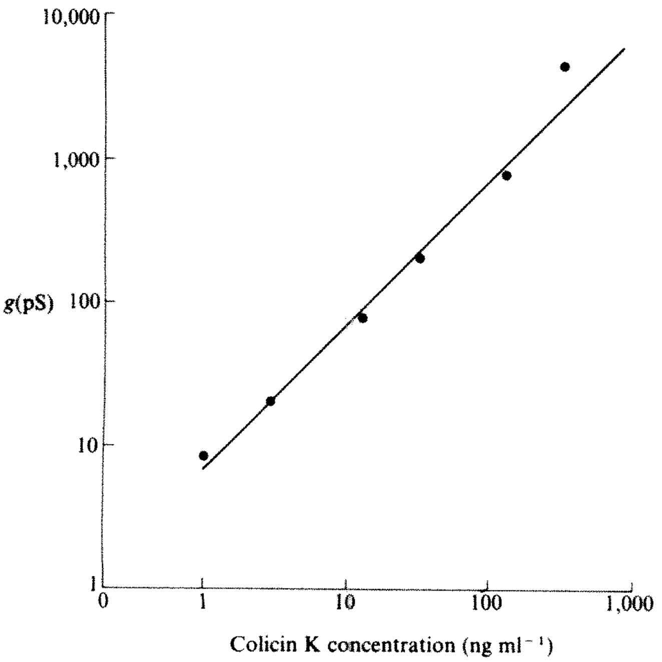

Fig. 4 Double logarithmic plot of steady-state, colicin K-induced conductance against colicin K concentration. The data were obtained on a single membrane at an applied voltage of −70 mV, with the colicin concentrations as indicated. (At this voltage the steady-state conductance has achieved a maximum value; thus the conductance plotted is the maximal conductance.) The line is drawn with a slope of 1. Conductance, and hence the number of channels, increases linearly with colicin K concentration, suggesting that each voltage-dependent channel is formed by one colicin molecule. The composition of the aqueous solutions is the same as for Fig. 1. Colicin K was from *P. mirabilis*.

A remarkable feature of colicin action is single-hit killing[8,18–20]. For colicin K, this feature arises because one poorly ion-selective channel of a few picosiemens conductance can deplete the cell of potassium, magnesium, and other small ions within a few minutes. We do not concern ourselves here with the details and sequence of events leading to cell death following changes in the internal ionic composition (for example, inhibition of macromolecule synthesis, loss of transport systems, drop in ATP levels[1,2]). Interestingly, however, *E. coli* survival following treatment with colicins K or E1 is greater in media containing high potassium and magnesium concentrations[38].

Depolarisation and disruption of active transport

A range of active transport systems are disrupted following treatment of cells with colicins of the E1 type[3,4,39]. This phenomenon is closely related to their ability to discharge the 'energised state' of the membrane[1–7], which occurs without induction of measurable proton flux[6,7,10]. According to the chemiosmotic theory, bacteria use the energy of oxidation to pump protons out of the cell[5]. The energy from the resulting membrane potential difference and *p*H gradient is used, together or separately, to drive a variety of active transport systems[5,40]. The energised membrane state may therefore be disrupted by discharging either the membrane potential or the *p*H gradient.

On the one hand, it is clear that the colicin channels described here would at least partially depolarise the membrane and hence disrupt active transport; the channels are poorly selective among cations and are significantly permeable to anions. Furthermore, numerous studies on bacterial cells and vesicles are consistent with membrane depolarisation[6,7,33,41–45]. On the other hand, given the low activity of protons in the medium (~10^{-6} M) and the enormous internal buffering capacity of the bacterial cell, the colicin K channel should not significantly dissipate any preexisting *p*H gradient as a primary effect. The internal buffering is such[46] that it would require the entry of enough protons to bring the internal concentration to 50–100 mM (assuming no buffering) to produce a change of one *p*H unit. Recent measurements of the *p*H gradient in cells exposed to colicins K and Ia are in agreement with this expectation[6,7,45].

Protection by uncouplers

The normal bacterial membrane potential[5] is of the proper sign and magnitude to open the colicin K channel. Cells are protected from colicin K action by treatments or agents (such as FCCP) which depolarise the membrane[16,18,21]. We suggest that channel closure at low membrane potentials (Figs 2 and 3) is the basis for this protection. (This, of course, does not explain protection from colicin E2 and E3 action by these agents[12,47].) One possible implication of our findings is that the colicin channel might depolarise the cell so severely that the channel would close. More definitive measurements of *in vivo* membrane potentials will be required to establish the likelihood of this possibility.

Colicin K and electrophysiology

The finding that colicin K creates voltage-dependent channels in lipid bilayer membranes opens a new and potentially important avenue of research into the physico-chemical mechanisms underlying the voltage dependence of channels in excitable membranes (for example, nerve and muscle). Colicin channels offer several advantages for investigation. (1) The protein can be highly purified in large quantities. (2) Genetic selection for modified molecules is possible; indeed, we have already noted that colicins K from *P. mirabilis* and *E. coli* differ somewhat both chemically and functionally. (3) The unidirectional orientation of colicin K in the membrane, as evidenced from the voltage dependence, makes possible one-sided modification of the colicin by standard methods of protein modification. This offers a potent technique for dissecting molecular function. (4) The availability of a series of bacteriocins that may form voltage-dependent channels similar to those created by colicin K permits a comparative study of channel formers. In addition to colicins E1 and Ia, which are already identified, other likely candidates are colicin Ib, colicin A, *Staphylococcus* bacteriocin 1580, colicin S.8 and *Serratia* bacteriocin JF246 (ref. 2).

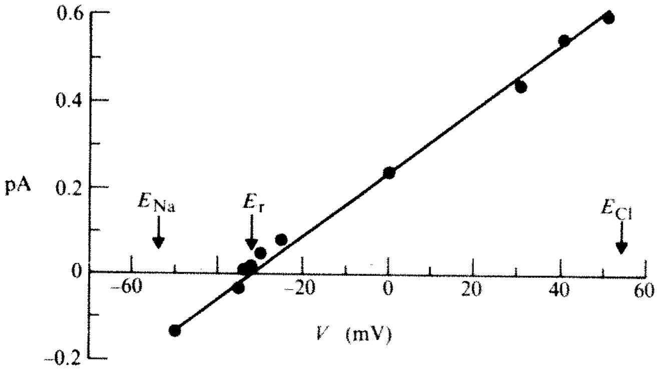

Fig. 5 Ion selectivity of the colicin K channel as determined from the reversal potential of individual channels. Across the membrane is a 10-fold gradient of NaCl concentration, which corresponds to about an 8.3-fold gradient in NaCl activity. The rear compartment contains 500 mM NaCl + 2 mM $CaCl_2$ + 1 μM EDTA + 2 mM MES (*p*H 6.1); the front (ground) compartment contains 50 mM NaCl + 2 mM $CaCl_2$ + 1 μM EDTA + 2 mM MES (*p*H 6.1). A very low concentration of colicin K from *E. coli* was added to the front compartment, and single-channel current jumps, such as shown in Fig. 3, were observed. The size of the current jumps is plotted as a function of membrane potential. The current jumps 'reverse' direction at $V = -33$ mV, closer to E_{Na} (≈ -54 mV), the sodium equilibrium potential, than to E_{Cl} ($\approx +54$ mV), the chloride equilibrium potential, thus showing that the channels are more permeable to Na^+ than to Cl^- by a factor of about 4.

We thank Dr K. Jakes for colicin E1 produced by *E. coli* W3110 (col E1), colicin E2 produced by *E. coli* E2TR2 (strain from P. Fredericq) and colicin E3 produced by *E. coli* W3110 (col E3); Dr S. E. Luria for colicin K produced by *E. coli* K235 and colicin E1 produced by *E. coli* LA779; Dr J. Konisky for colicin Ia produced by *E. coli* W3110 (col Ia − CA53); and Dr M. Jesaitis for colicin K produced by *P. mirabilis*. We also thank Drs Jakes and Luria, and Mr M. Weiss for discussions. S.J.S. was supported by NINCDS postdoctoral research fellowship NS 05799-01 and an Alfred P. Sloan Foundation Research Fellowship BR 189S. B.L.K. and A.F. were supported by NIH grants NS 14246-01 and 5T5GM1764.

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letters to nature

Space density evolution of quasars

ATTEMPTS to obtain interesting cosmological information from the study of quasars have been thwarted by the apparent large spread in quasar luminosities so that the conventional Hubble plot produces a scatter diagram[1]. Several authors[2–4] have used an absolute magnitude–redshift diagram to provide a standard candle and hence improved the Hubble diagram. However, little work has apparently been done on using the $(M, \log z)$ plot as a cosmological indicator in its own right, in place of the Hubble diagram. It is shown here that although evolution may be zero, the uncertainty in the quasar luminosity function allows an upper limit on the quasar space density.

Figure 1 shows the $(M_v, \log z)$ plot of quasars from the catalogue of Burbidge *et al.*[5], which were chosen to have $z \leq 2$ and good photometry. The following assumptions have been made. First, quasar redshifts are of cosmological origin so that redshift is a valid indicator of distance, thus[6]

$$m = M + 5 \log D(z) + \text{const} \tag{1}$$

$$D(z) = \frac{1}{q_0^2}\{q_0 z + (q_0 - 1)((1 + 2q_0 z)^{1/2} - 1)\} \tag{2}$$

Second, the Universe can be modelled by a zero cosmological constant, zero pressure Friedmann cosmology. Third, $q_0 = 1$ has been assumed for Fig. 1, but this will be shown not to be essential.

Figure 1 shows that the luminosity of the brightest object at a given redshift is a smooth function of redshift, as has been observed by others[2,3,7]. We propose that the upper envelope is due to a statistical sampling effect identical to the Scott effect[8], which is, as the number of objects in a sample chosen from a given luminosity function increases, the brightest object moves towards the bright end of the distribution, thus as z increases the volume of space sampled increases, as does the number in the enclosed sphere, so that the luminosity of the brightest object in the sphere can be expected to increase. This has been suggested recently by Sramek and Weedman[7]. This theory therefore provides a functional relationship between the luminosity of the brightest object and redshift, which should reproduce the upper envelope in the $(M, \log z)$ plot, given by

$$L_{\max}(z) = f(N_z) \tag{3}$$

The model is characterised by the following assumptions: (1) the space density of quasars is constant at a given epoch, that is the cosmological principle holds. (2) Quasars are distributed according to the normalised luminosity function

$$N(M)\,\mathrm{d}M = N_0 \gamma\, \mathrm{e}^{\gamma M}\, \mathrm{d}M \tag{4}$$

where N_0 is the number in the sample, M is the absolute magnitude and γ is a parameter to be determined observationally. This exponential function in magnitude is equivalent to a power law function in luminosity, and has been discussed elsewhere[8–11]. (3) If N_0 objects are chosen from a distribution $\mathrm{e}^{\gamma M}$ then the value of M for the brightest object, $M_{\max}$, is given by

$$N_0 = \mathrm{e}^{-\gamma M_{\max}} \tag{5}$$

This result can be derived as follows. The normalised distribution in $N_0 \gamma\, \mathrm{e}^{\gamma M}$. It is now postulated that the brightest object is situated where $\int_{M_{\max}}^{-\infty} N_0 \gamma\, \mathrm{e}^{\gamma M} = 1$, that is where the number of objects expected in the tail of the distribution is one, from which equation (5) follows. (4) If density evolution exists it can be characterised by

$$\rho(z) = \rho_0\, \mathrm{e}^{\alpha\tau} \tag{6}$$

where τ is the look-back time to redshift z and ρ_0 is the local

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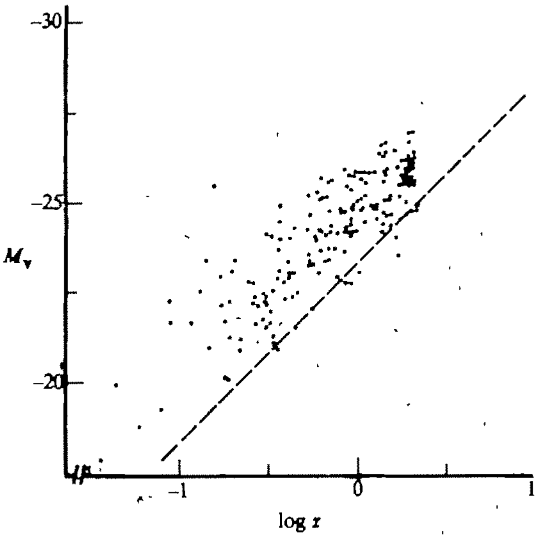

Fig. 1 Absolute magnitude of Burbidge quasars for $q_0 = 1$, as a function of log z. Dotted line shows $m_v = 19$ locus.

density. This exponential law has been discussed by several authors[9,10,13].

The model therefore gives

$$M_{\max} - M^0 = -\frac{1}{\gamma}\ln N(z) \qquad (7)$$

with

$$N(z) = \int_0^z \rho(z)\,\mathrm{d}V \qquad (8)$$

where M^0 is a luminosity cut-off to avoid divergences. The volume of space out to z is calculated from the equations given in ref. 6.

From equation (7) it follows that for low redshift, where cosmological and evolutionary effects are small

$$M_{\max} - M^0 = -\frac{1}{\gamma}\ln \rho_0 \frac{4}{3}\pi\left(\frac{zc}{H_0}\right)^3 \qquad (9)$$

assuming the linear Hubble law, $R = zc/H_0$. Thus the upper envelope in the $(M, \log z)$ plot has a gradient $-6.9/\gamma$ at low redshift. Hence the slope at low z is determined by the index of the luminosity function. Several values of luminosity function index have been given ranging from the high value[7,9] $\gamma = 1.7$ to the low value of 1.1 (ref. 11). For the present purposes, a value of 1.4 ± 0.3 has been taken. It is to be expected that the luminosity function will be improved on in the future.

Fig. 2 Data from Fig. 1 with theoretical upper envelopes superimposed for $\alpha = 0$, 2 and 10 (see text for definition of α).

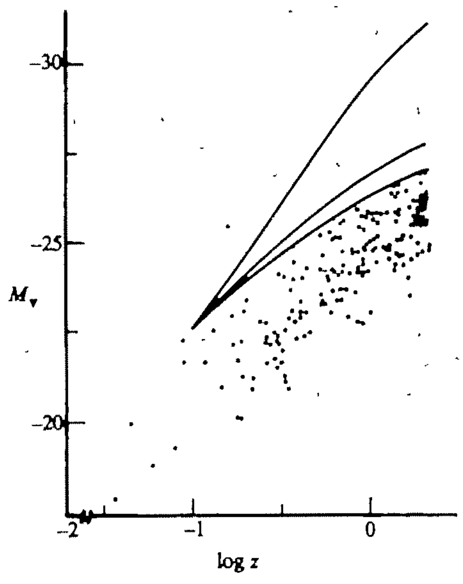

Figure 2 shows the data of Fig. 1 with three theoretical curves superimposed. The lower curve is that for zero evolution, and it can be seen that this calculation reproduces well the observational upper envelope. If a different value of the luminosity function index γ is used, it will be necessary to introduce space density evolution, as discussed by Setti and Zamorani[11]. Thus the uncertainty in γ leads to an uncertainty in evolution. The result obtained for the rate of space density evolution for $q_0 = 1$ is $\alpha = 0 \pm 2$.

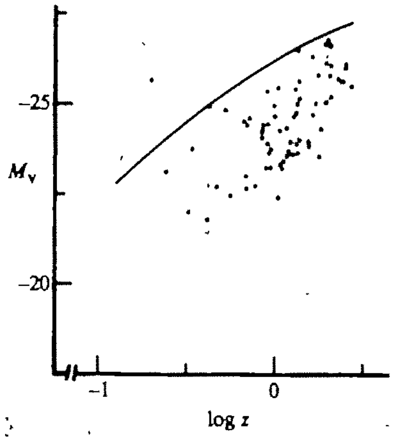

Fig. 3 Absolute magnitude of complete sample of Parkes quasars for $q_0 = 1$, as a function of log z. The theoretical upper envelope for $\alpha = 0$ (no evolution) is superimposed

Table 1 compares the upper limit $e^{2\tau}$ with other evolution laws, where it can be seen that the present result gives much slower evolution. The effect of evolution on the upper envelope is shown in Fig. 2 for $\alpha = 2$ and 10, the latter being comparable to the $10^{5\tau}$ law of Schmidt[9,10]. It seems important that if the luminosity function is as flat as found by Setti and Zamorani ($\gamma = 1.1$) then evolution in a negative sense is implied, that is fewer objects in the past, but still at a slow rate. The dependence of the best-fit value of α on q_0 is:

q_0	0	0.5	1	2
α	1	0.5	0	−0.5

It can be seen from equation (9) that the zero point of the theoretical curve is determined by H_0 and ρ_0, the local space density quasars. Assuming $H_0 = 50$ km s^{-1} Mpc^{-1} this leads to a value of ρ_0, which has been given in Table 2, where $\rho_0(> M^0)$ is the space density of objects brighter than M^0 and ρ_0 diff. is the differential luminosity function scaled to the local space density, that is the number of objects per Gpc^3 with magnitude in a unit range about M^0. The results agree with those of Sramek and Weedman to within a factor of 2, at the bright end.

The Scott effect, which has been the basis of this letter, has had an important influence on previous determinations of quasar evolution. Apparently the Scott effect will mimic evolution by providing an excess of luminous objects at high redshift, thereby biasing the V/V_M test in exactly the same way as evolution. The effect will also show up in number–magnitude counts by simulating a 'local hole'[12]. Hence it seems that what has previously been interpreted as evolution is just the result of the statistical space-sampling effect discussed above.

A further point is the behaviour of the curve beyond $z = 2.5$, where the observed number of quasars is very low (Smith[14]). As well as the important fact that space volume is a very low function of z, so that there is very little space volume between $z = 2.5$ and 3, it now seems that the space density is much lower than previous evolution laws have implied, which makes the expected number of objects even smaller. In addition, as Fig. 2 shows, the absolute magnitude of the brightest quasar remains nearly constant beyond $z = 2.5$, so that the objects apparently become increasingly faint with increasing redshift. This situation has been discussed by Carswell and Smith[15] who found that the observed number of quasars of high redshift can be accounted for assuming zero evolution. Thus it seems that the era of quasar formulation has not yet been found.

Table 1 Evolutionary laws

z	τ	$(1+z)^6$	$10^{5\tau}$	$e^{2.0\tau}$
0	0	1	1	1
0.2	0.269	3	22	1.7
0.5	0.495	11.4	298	2.7
1	0.683	64	2,600	3.9
2.0	0.832	729	14,400	5.3
3.0	0.892	4096	28,840	6.0
∞	1	∞	100,000	7.4

The Burbidge catalogue of QSOs has been used in the present application of this technique because it is the largest body of data available. However, due to the heterogeneity of this catalogue, selection effects might be important. It is difficult to see where such effects might arise as they would have to be redshift-dependent to affect the present analysis and because most of the objects used are radio sources, redshift dependent effects should be negligible. In addition, redshift dependence in the optical identification is unlikely, as it is the brightest objects at each redshift which are of concern and the apparent magnitude of these changes only slowly with redshift, in the redshift range used here. In any case, it was felt important to test the method on a complete and homogeneous set of data. Figure 3 shows the (M_v, log z) diagram for Parkes objects from the Wills and Lynds[13] catalogue, where it can be seen that the result derived from Fig. 1 is unchanged. In this work k-corrections have been neglected as they are expected to be small[16]. A larger sample of low redshift ($z \leqslant 0.1$) quasars would be very valuable to improve the determination of slope and zero-point. It has been suggested[17] that steep and flat radio-spectrum quasars differ in evolutionary properties, and the present approach should be able to discriminate between the two species on those grounds, provided a good enough sample of data can be acquired.

We have found that previous determinations of quasar space density are subject to a statistical sampling effect depending on space volume. The present result is that evolution may be zero but the uncertainty in the quasar luminosity function allows an upper limit of $\rho(\tau) = \rho_0 e^{2\tau}$.

That the present method works at all must favour the cosmological nature of quasar redshifts. Also it is predicted that absolute magnitude of the brightest object at a given redshift increases very slowly beyond $\sim z = 3$ so that objects beyond that become increasingly faint and more difficult to detect.

Table 2 Local space density of quasars

M^0	log F (S.W.)	$\rho_0(M^0)$ (Gpc^{-3})	ρ_0 diff	ρ_0 (S.W.)
−25.9	44.4	9.1×10^{-3}	1.4×10^{-2}	0.022
−24.9	44.0	4.1×10^{-2}	6.1×10^{-2}	0.117
−23.9	43.6	1.8×10^{-1}	2.7×10^{-1}	0.58
−22.9	43.2	8.2×10^{-1}	1.2	3.47
−21.9	42.8	3.7	5.5	20.9
−20.9	42.4	16.4	24.6	117
−19.9	42.0	73.4	110	741

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Micropulsations observed by whistler-mode transmissions

DUCTED whistler-mode signals from VLF transmitters are typically Doppler shifted by ~0.1 Hz from the transmitter frequency. This Doppler shift has been attributed to motion of the ducts (geomagnetic field-aligned enhancements of plasma density) and measurements of the Doppler shift of NLK (18.6 kHz) signals have been used to observe relatively steady duct drifts over several hours[1,2], as well as duct oscillations in the Pc4–5 range[3]. We report here duct oscillations in the Pc3 range of a 6.6 kHz signal from a transportable VLF transmitter in Alaska, which was received at Dunedin, New Zealand[4]. Two events were observed, about six hours apart, as shown in Figs 1 and 2. For both these events, comparisons between the Doppler oscillations and ground magnetograms fit very well with current ideas about micropulsations.

Strong whistler-mode signals were received on 10 September 1973 from 13.00 to 13.30 UT showing both linear and nonlinear amplification as reported elsewhere[5], and from 19.40 to 20.30 UT where only linear amplification occurred. Various transmission formats were sent, and the received phase at Dunedin was compared with an accurate reference phase using a 'phasogram'[5]. This is a plot, made on moving film, of phase difference against time. Thus by measuring the slope, the frequency offset, Δf, is obtained as a function of time.

Figure 1*a* shows this frequency offset for the interval 13.14–13.27 UT. A sinusoidal function was fitted to the data by the method of least squares, giving a period of 94.0±0.4 s. At 13.00 UT one-hop delays were measured to be 1.500 and 1.455 s (ref. 5) indicating transmission in two closely spaced ducts. Dispersion analysis of whistlers occurring simultaneously with the above group delays showed the two ducts were at $L = 3.88$ and $L = 3.77$. However, during the period analysed only the signal with the 1.455 s delay was apparent, indicating that the observed oscillations occurred along the $L = 3.77$ field line.

Figure 1*b* shows an enlarged portion of the **H** trace from a magnetogram recorded at Campbell Island ($L = 4.0$), which is situated near the foot of the $L = 3.8$ field line (**H** is much stronger than the **D** component). Pulsations of 94 s period with amplitude ~1.3 nT are clearly present (all amplitudes are mean-to-peak).

Magnetic field measurements in the form of $|\partial \mathbf{B}_z/\partial t|$ were also made at Dunedin ($L = 2.7$) by recording, on the same magnetic tape as the VLF data, the voltage induced in a horizontal loop[6] of $\sim 5\times 10^4$ m^2. This enabled accurate synchronisation between magnetic and VLF data. A very weak 94 s pulsation was found by spectral analysis.

As the periods of the Doppler and magnetic oscillations were the same, the oscillation was meridional and most likely fundamental with an antinode of motion at the Equator[3]. This is supported by calculations of the resonant frequency of oscillating field lines in a dipole field and variable plasma density[7], where the fundamental period at $L = 3.8$ (colatitude = 30.9°) is ~90 s.

The phase path and hence phase time, T_p, varies with latitude, tube content, and maximum equatorial displacement of the duct from its mean position, caused by the distortion of the dipole field. Andrews *et al.*[8] showed that the electron content of the duct remains constant for Doppler fluctuations with periods <30 min. Hence we can assume that the Doppler shift is entirely due to duct movement (where magnetic field strength, and hence refractive index and path length, change). The Doppler oscillation is assumed to be due to the radial component of motion because the azimuthal component has little effect on the phase path[3].

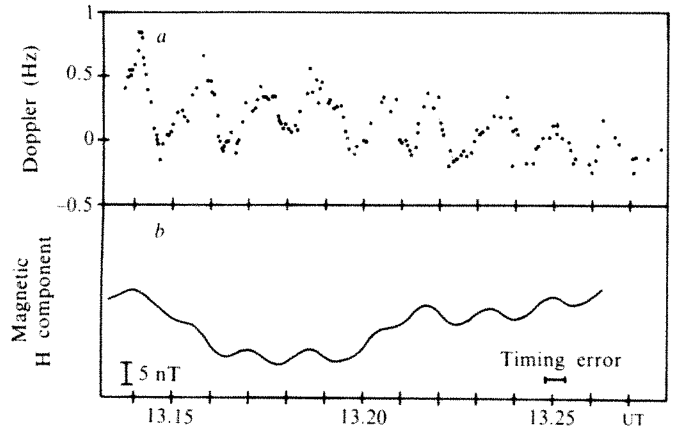

Fig. 1 *a*, Doppler shift of 6.6 kHz whistler-mode signal plotted against time for 13.14–13.27 UT, 10 September 1973. The Doppler shift is obtained by phase analysis as described in ref. 5. *b*, Magnetic **H** component scaled from a Campbell Island ($L = 4$) magnetogram. Absolute timing is ±15 s on the magnetogram. Magnetic variation $|\partial \mathbf{B}_z/\partial t|$ at Dunedin also shows a very weak 94 s pulsation.

The rate of change of phase time, dT_p/dt, equals the fractional change in frequency, $-\Delta f/f$. In its effect on the VLF wave, a small change in equatorial displacement, d (with a node near the Earth's surface) is similar to a small change in L-value of the duct in an undistorted dipole field, as the whistler-mode wave spends most of its time near the Equator. Thus by calculating the variation of phase time with latitude in a suitable model magnetosphere, we can calibrate dT_p/dt in terms of an equatorial duct velocity, v_0, given by:

$$v_0 = R_0 \frac{dL}{dt} = -R_0 \frac{dL}{dT_p}\frac{\Delta f}{f}$$

where R_0 = Earth's radius. The peak displacement, d, is obtained by integrating v_0 over one quarter period. A model magnetosphere with a steep equatorial electron density profile proportional to L^{-5}, as the duct is near the plasmapause, gives $dT_p/dL = -0.34$ s and $d \sim 16$ km. An estimate of the wave magnetic field can be obtained from this using a simplified two-dimensional model of a transverse standing Alfvén wave. The wave magnetic field, $\mathbf{b}_x$, in the North–South plane above the ionosphere is given by:

$$\mathbf{b}_x = \mathbf{B}\frac{\partial \xi_x}{\partial z}$$

where $\mathbf{B}$ = local magnetic field, ξ_x is the meridional displacement and z is distance along a field line from the ionosphere. Assuming a dipole field and that $\mathbf{b}_x$ varies sinusoidally along the field line, with a node at the Equator, we get a displacement of 7.9 km nT^{-1} at $L = 3.8$. Hence $\mathbf{b}_x$ is just above the ionosphere (1,000 km) ~ 2 nT. This is slightly greater than the **H** component on the Campbell Island data.

Note that the Doppler oscillation is superimposed on a slowly varying Doppler shift. If this mean shift is also due to duct drift, then perhaps the damping of the oscillation in Fig. 1*a* can be interpreted as spatial attenuation as the duct drifts inwards from the resonant latitude. Time damping is unlikely as the Campbell Island data show no such damping in Fig. 1*b*. From the least-squares fit to Fig. 1*a*, the time for the amplitude to be halved is 408 s. The average Doppler shift over this period gives a net equatorial drift velocity of 0.45 km s^{-1}, so the half-width of the resonance region is ~180 km. This supports models with a sharp amplitude peak at a particular latitude[9].

The one-hop group delay for the signal in Fig. 2*a* was 1.150 ± 0.005 s which, when combined with whistler analysis gives the duct location at $L = 2.85 \pm 0.02$. Note that the frequency offset is much smaller than in Fig. 1*a*; the period of oscillation is 60.3 ± 0.5 s. Magnetic variation at Dunedin Fig. 2*b* shows pulsations of this period and of about 0.2 nT amplitude very clearly. The Campbell Island magnetogram also showed barely resolved 60-s oscillations of about 0.4 nT amplitude on the **H** trace.

Calculations using a magnetospheric model with a typical equatorial density variation proportional to L^{-4} gave $dT_p/dL = -0.53$ s at $L = 2.85$, implying a peak displacement of ~1 km for the field line oscillation. This corresponds to a 0.4-nT disturbance just above the ionosphere.

Resonant field line calculations show[7] that, for a fundamental oscillation of 60 s period, $L \approx 3.4$. Hence this observation is from below the resonant latitude and is consistent with the relatively low amplitude observed in the magnetosphere.

Cross-correlations of the digitised Dunedin magnetic variation $|\partial \mathbf{B}_z/\partial t|$ with Doppler shift for both intervals (Fig. 3*a*) show they are in phase quadrature. (Timing accuracy at Campbell Island was insufficient to allow phase comparisons with Dunedin.) The wave in the resonance region is most likely to be linearly polarised as Campbell Island **H** and **D** components seemed to be in phase. We assumed circular polarisation[3] for the Dunedin magnetic data as it was far from resonance in both cases. Theoretical calculations show[9] that the phase of $\mathbf{b}_x$ above

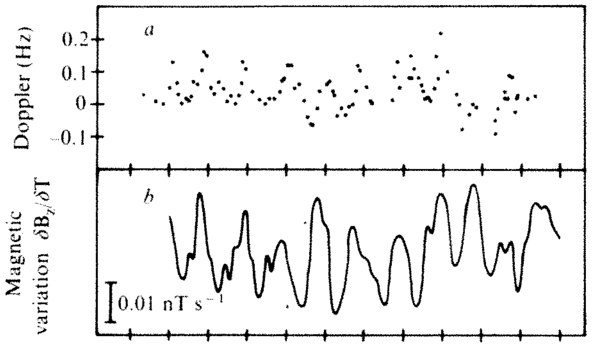

Fig. 2 *a*, Doppler shift observed in the interval 19.44–19.54 UT, 10 September 1973. Note the smaller amplitude than in Fig. 1*a*. *b*, Magnetic variation $|\partial \mathbf{B}_z/\partial t|$ recorded at Dunedin simultaneously. Pulsations of ~0.2 nT are very clear whereas they are barely resolvable on the Campbell Island **H** trace (<0.4 nT).

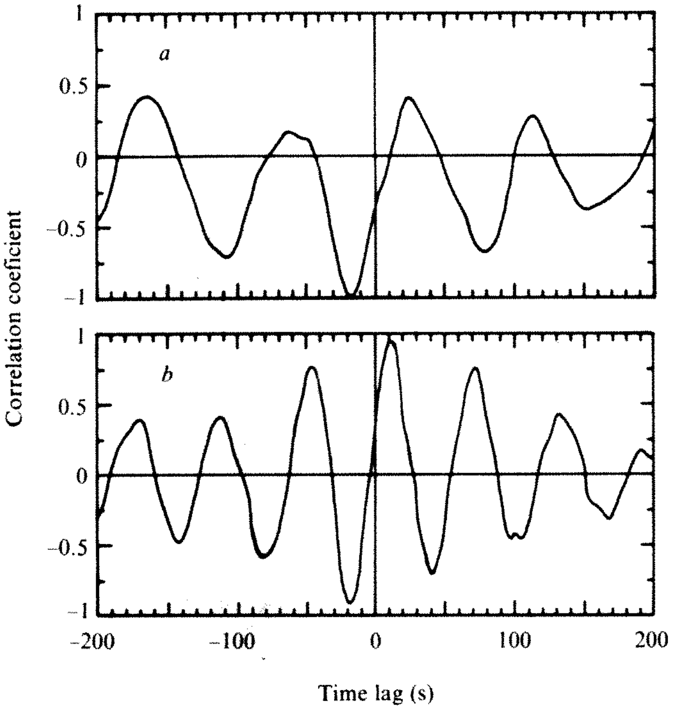

Fig. 3 Cross-correlation between Doppler shift (Δf) and magnetic variation $|\partial \mathbf{B}_z/\partial t|$ recorded at Dunedin. Positive lag means magnetic variation leads Doppler shift. *a*, For the interval 13.14–13.27 UT a lag of 24 s is present, equal to a quarter period. *b*, The lag is 10 s for the interval 19.44–19.54 UT.

the ionosphere is relatively constant with latitude including the resonant latitude. Hence the cross-correlations are consistent with a phase rotation of 90° through the ionosphere as f and $|\partial \mathbf{B}_z/\partial t|$ are in phase quadrature with $\mathbf{b}_z$ and $\mathbf{B}_z$ respectively. This assumes $\mathbf{B}_z$ has mainly the phase of the **H** component.

The above observations show that by phase tracking whistler-mode signals, duct oscillations in the Pc4 (and probably Pc3) range can add new information to existing ground (and satellite) micropulsation data. Indeed the fine latitudinal structure of the resonance region in the magnetosphere could not be easily obtained by other means in these latitudes. At high latitudes ($L \sim 6.3$) this structure has been studied by auroral radar[10] and estimates of the width of the resonance region in terms of $\Delta L/L$ are about four times (A. D. M. Walker, in preparation) our estimate for $L \sim 3.8$.

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Triplet state resonance Raman spectroscopy

AN important objective of theoretical photochemistry is to describe the interplay between the different decay channels of molecules in electronic excited states[1,2]. Detailed information on the potential energy hypersurface is required to specify the molecular dynamics in terms of saddlepoints and activation energies along the reaction coordinate. Excited electronic states differ from the ground state because of the changes in the electronic distribution within the molecular framework. These changes are accompanied by changes in bond orders, force constants of vibration and dissociation energies. Vibrational spectra of excited states could be expected to provide important information on changes in frequencies of particular normal modes relative to those of the ground state molecule. Triplet excited states in solution have been studied by many techniques, the most common being flash photolysis in combination with optical absorption spectroscopy. Vibrational spectroscopy has to our knowledge only been reported in a single case, where one vibrational band of the lowest state of deuterated naphthalene generated by steady-state illumination was detected by infrared spectroscopy[3]. Recently, resonance Raman spectroscopy has been established as a powerful technique for studying shortlived species in solution[4]. This technique combines high sensitivity and time resolution with selectivity and structural information. It has been used successfully to study free radicals in solution generated by stopped flow methods[5], electrochemically[6], by pulse radiolysis[4] and recently by laser flash photolysis[7]. Here we make the first report on the resonance Raman spectrum of a molecule in its triplet state generated by pulse radiolysis.

The experimental system has been described previously[4]. Excited states were produced by electron irradiation with a pulse of high energy electrons. Concentrations and kinetics of the transient species were established by optical absorption spectroscopy, and a pulsed dye laser and an optical multichannel analyser coupled to a computer were used to record the resonance Raman spectrum of the transient species.

It is well established[8] that the lowest excited triplet state is formed on electron irradiation of solutions of organic molecules in benzene. The mechanism proposed is the formation of a radical cation–electron pair of a solvent molecule followed by fast recombination into a low-energy excited state, and the subsequent energy transfer to solute molecules exciting these into the lowest triplet state. We investigated a solution of 0.01 mol dm^{-3} of p-terphenyl in benzene. The maximum of the triplet–triplet absorption spectrum was found at 460 nm which agrees with the literature[9], and the wavelength of the dye laser was tuned to approximately this value. The lifetime of the

Table 1 Vibrational wavenumbers (cm^{-1}) of p-terphenyl in its ground state and lowest excited triplet state

Symmetry species in D_{2h}*	Ground state†	Triplet state
A_g	1,613	
		1,540
	1,599	
A_g	1,505	
	1,494	
A_g	1,284	1,350
A_g	1,195	1,227
A_g	1,038	
A_g	1,008	
		921
	993	
A_g	761	
	593	587

* According to ref. 10. † In CS_2, CCl_4.

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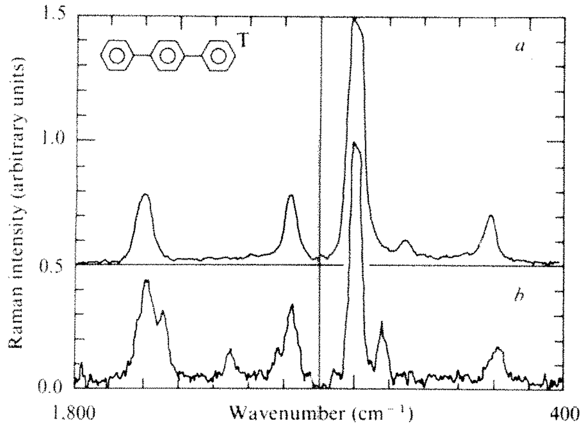

Fig. 1 *a*, Raman spectrum of a 0.01 mol dm^{-3} solution of *p*-terphenyl in benzene; *b*, resonance Raman spectrum of the same solution 600 ns after irradiation with a 30-ns pulse of 2 MeV electrons. Each spectrum recorded with two laser pulses (as indicated by the vertical line), excitation wavelength 459.0 nm.

excited species was 4.4 μs. The concentration of excited states was estimated to be $\sim 1 \times 10^{-4}$ mol dm^{-3}.

The resonance Raman spectra without and with electron irradiation are shown in Fig. 1. Table 1 lists the Raman bands found in the parent *p*-terphenyl molecule and the transient excited state. Characteristic wavenumber shifts occur in going from the parent molecule to the excited state species. Tentative assignments can be made according to the normal coordinate analysis for biphenyl by Zerbi and Sandroni[10].

All bands of the transient species, with the single exception of the 587 cm^{-1} band, are assigned to totally symmetric vibrations, suggesting that A-term scattering[11] predominates. This seems to indicate a substantial change in geometry in going from the lowest to the higher excited triplet state. According to Zerbi and Sandroni the main contribution to the Raman band of the parent molecule at 1,284 cm^{-1} originates from the ring–ring C–C stretching vibration. We believe that this band is shifted upwards by 66 cm^{-1} in the spectrum of the triplet species, which suggests an increasing bond order and a higher degree of planarity in the triplet state compared to the ground state. This result agrees with the literature: Orloff and Brinen[12] conclude from MO calculations of zero-field splitting parameters that there is a striking change in geometry in going from the ground state to the lowest triplet state of the polyphenyl-benzenes, and that a substantial shortening of the ring–ring bond occurs. Wagner[13] suggests on the basis of spectroscopic and quenching results that in the ground state biphenyl is twisted with an interplanar angle of 25°, whereas the triplet state seems to be planar.

We have demonstrated that triplet states can be investigated by resonance Raman spectroscopy. Because of the obvious advantages of this technique compared with absorption spectroscopy, it is expected that it will become a valuable tool in the investigation of shortlived excited states, their structure and kinetics.

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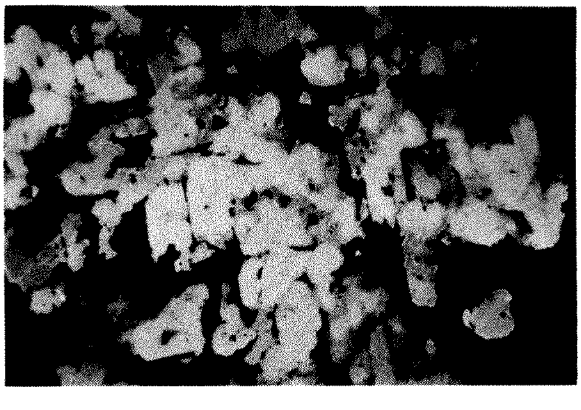
FeNi superlattice formation by corrosion of Santa Catharina meteorite

KNUDSEN *et al.*[1-3] have recently presented evidence from Mossbauer spectroscopy and X-ray diffraction, that an FeNi L1_0 superlattice exists in the taenite of the Cape York and Toluca meteorites. This superlattice, which had previously only been reported in neutron irradiated specimens[4], is thought to have formed during the extremely slow cooling, 1°C Myr^{-1}, of the parent bodies of these meteorites. Here we report briefly on the discovery of the FeNi superlattice in a nickel-rich ataxite, Santa Catharina, and show that in this meteorite the ordering occurred during terrestrial corrosion.

Apart from some large idiomorphs of iron nickel phosphide (schreibersite) and a dispersion of small plates of the phosphide (rhabdite), the Santa Catharina meteorite contains two main microstructural constituents, one of which, the dark etching constituent of Fig. 1, is a face-centred cubic (f.c.c.) taenite containing 30.7 atom%Ni and having lattice parameter $a =$ 3.586 Å. The structure of the light etching constituent has, until now, been uncertain. Thermomagnetic measurements[5] indicated it to be a 55 atom%Ni taenite, but microprobe analysis[6] showed that it contained up to 25 atom% oxygen. We have established that this light etching constituent consists of a dispersion of particles of FeNi spinel, Fe_2NiO_4, ~50 Å thick, in an ordered 53 atom%Ni taenite. The spinel particles comprise between one- and two-thirds (atom fraction) of this constituent. They are, however, too small to diffract X rays coherently and diffraction patterns from this constituent are single crystal patterns from the taenite. Patterns obtained using $Co_{K\alpha}$ radiation contain reflections from all of the {100}, {210}, {211} planes and from planes with higher indices. These reflections indicate that domains of all three orientations of the superlattice, FeNi, are present. The lattice parameter, 3.582 Å, of the ordered taenite is nearly equal to that of the 30.7 atom%Ni taenite and its orientation is the same. The spinel particles can be detected in

Fig. 1 Light and dark etching constituents in the Santa Catharina meteorite. Parts of some specimens consist almost wholly of the dark constituent. Nital etch. ×200.



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extraction replicas, by electron microscopy and electron diffraction. They coarsen rapidly on heating and can be resolved optically after 1h at 615 K. Reflections from the spinel particles appear in the X-ray diffraction patterns of specimens heated at 575 K for 1 h.

The terrestial origin of the light etching constituent has been established by observing its formation in samples consisting almost wholly of the 30.7 atom%Ni taenite that were cut from the meteorite and subjected to corrosion at room temperature in water containing a small concentration of chloride ions. Figure 2 shows a section through the light etching constituent produced in a specimen corroded for 35 d. It has been detected in specimens corroded for as little as 5 d. The constituent produced by corrosion is the same in all respects as the natural material; the taenite is ordered and the compositions and electron microstructures are the same.

The mechanism by which this corrosion product is formed will be discussed in more detail elsewhere, but it is evident that the enrichment of the taenite from 30.7 to 53% Ni and the ordering of this phase, requires a degree of atomic mobility not normally available at room temperature. It is likely that this enhanced mobility is attributable to di-vacancies generated at the surface by the preferential dissolution of iron atoms[7]. Such excess vacancies would also permit the ingress of oxygen atoms to form the spinel.

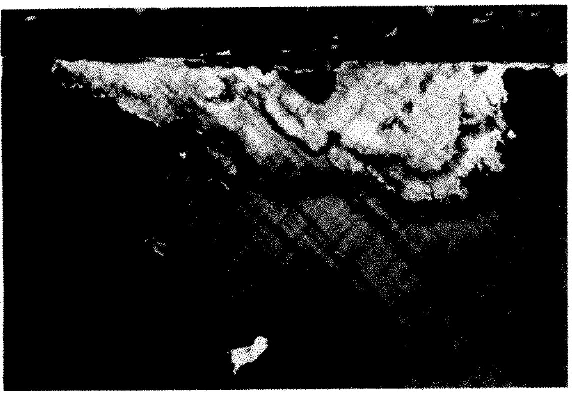

Fig. 2 Light etching constituent produced by corrosion. Nital etch. ×200.

The formation of the $L1_0$ superlattice during corrosion obviously means that care must be taken when using the presence of this superlattice as an indication of the thermal history of meteorites. However, it is not expected that the taenite lamellae in octahedrites would be susceptible to this type of corrosion process while the low nickel kamacite is present. Even when the kamacite has corroded away the high nickel concentrations, which are produced in the outer layers of the taenite during the precipitation of the kamacite, would corrode much more slowly than the taenite of Santa Catharina.

It is curious that the lattice parameter of the nickel-rich ordered taenite in Santa Catharina is the same as that measured by Knudsen *et al.* in Cape York and Toluca. In these meteorites, the taenite lamellae were found to contain a disordered f.c.c. phase as well as the superlattice, the lattice parameters of both phases being 3.582 ± 0.002 Å. Comparison with published parameters[8] showed that the disordered phase contains approximately 28 atom%Ni and it was proposed that these two phases occur in varying proportions in the nickel concentration gradient in the taenite. The concentration gradient is considered to be 'frozen in', but within each volume element short-range diffusion of nickel has permitted the development of the two-phase structure. In this model the two-phase region is identified with the dark etching zone of so-called cloudy taenite observed in many octahedrites, whereas the clear taenite rim is considered to consist almost wholly of superlattice.

Knudsen *et al.* consider that the domains of ordered taenite in the two-phase region have the composition FeNi, but published lattice parameters of iron nickel alloys indicate that ordered taenite with $a = 3.582 \pm 0.002$ Å contains 53 ± 2 atom%Ni. Our microprobe analyses of Santa Catharina confirm that ordered taenite with this lattice parameter contains more than 50 atom%Ni. These analyses indicate that the light etching constituent contains varying proportions of Fe_2NiO_4 and taenite containing ~55 atom%Ni.

It is surprising that a single lattice parameter was observed in the Ni concentration gradients of Cape York and Toluca. The nickel concentration in the taenite at the taenite–kamacite interface is determined by the composition of disordered taenite in equilibrium with kamacite at the temperature at which growth of the kamacite ceased. The concentration of nickel in ordered taenite in metastable equilibrium with disordered 28 atom% Ni taenite is expected to be below this upper limit. Thus, the concentration gradient should contain a two-phase region in which the lattice parameter of the ordered taenite is constant, and a single-phase region, in which the lattice parameter varies between this value and that of taenite in equilibrium with kamacite. The range of nickel concentrations in this single-phase region indicated by the error limits of the parameters measured by Knudsen *et al.* is 53 ± 2 atom%Ni, implying that the lower limit of the clear taenite is at 51 atom%Ni. This conclusion conflicts with other measurements[9,10] which indicate a lower limit of 40 atom%Ni.

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Earthquake precursors from Earth tilt observations corrected for rainfall

AN Earth tilt meter at Wellington, New Zealand (lat 41°14′S, long 174°47′E) has been operating since 1972 in old underground tunnels which are about 15 m below the surface. Installation transients, at first of the order of several microradians per year, have decayed slowly and are now about 0.3 μrad yr^{-1}. As is well known[1,2] rainfall and evaporation, in all but very deep sites, usually completely obscure any obvious relationship with tectonic events. Wood and King[3] have made one unsuccessful attempt to deal with this problem but the largest earthquake occurring was only of magnitude 5.2. A new approach, described here, has yielded significant correlations of tilt with two, and possibly three, earthquakes of magnitudes 5 to 6.2. The precursor times are in fair agreement with Rikitake's[4] equation.

Our approach has been to deal solely with monthly mean values of tilt, rainfall and the other meteorological elements involved. After correcting for both the exponential installation transient and average seasonal variation the monthly tilt departure from normal, T_0, correlates significantly with the monthly

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rainfall minus evaporation departures, $(R-E)$, for the previous 5 months. This leads to a tilt correction of the form:

$$T\alpha = a(R-E)_0 + b(R-E)_1 + c(R-E)_2 + d(R-E)_3 + e(R-E)_4 + f(R-E)_5 + gt_0 + ht_1 + jP_0$$

where the suffix refers to the nth month before the one in question, t is the monthly mean temperature departure and P that for atmospheric pressure. Meteorological data were supplied by a nearby weather station.

The coefficients a–h were optimised to produce the highest correlation coefficient between the values T_α and T_0. Other possibilities were also tried, like extending the series for more than 5 months into the past, but the highest correlation coefficients obtainable were $r = 0.89$ and 0.83 for the N–S and E–W tilt components respectively. This was done for the entire period of the study, 1974–78, and indicates, for instance, that only $100(1-r^2) = 21\%$ of the N–S variations observed are due to non-meteorological causes, presumably tectonic.

The residual tilt, $T = T_0 - T_\alpha$, is shown in Fig. 1, together with earthquakes of $M \geqslant 5$ occurring near enough to the station to be of significance. Figure 2 shows the epicentres, magnitudes, and dates of all normal earthquakes $M \geqslant 5$ occurring within about 150 km during the period analysed. The NZ Seismological Observatory supplied the seismological data[5].

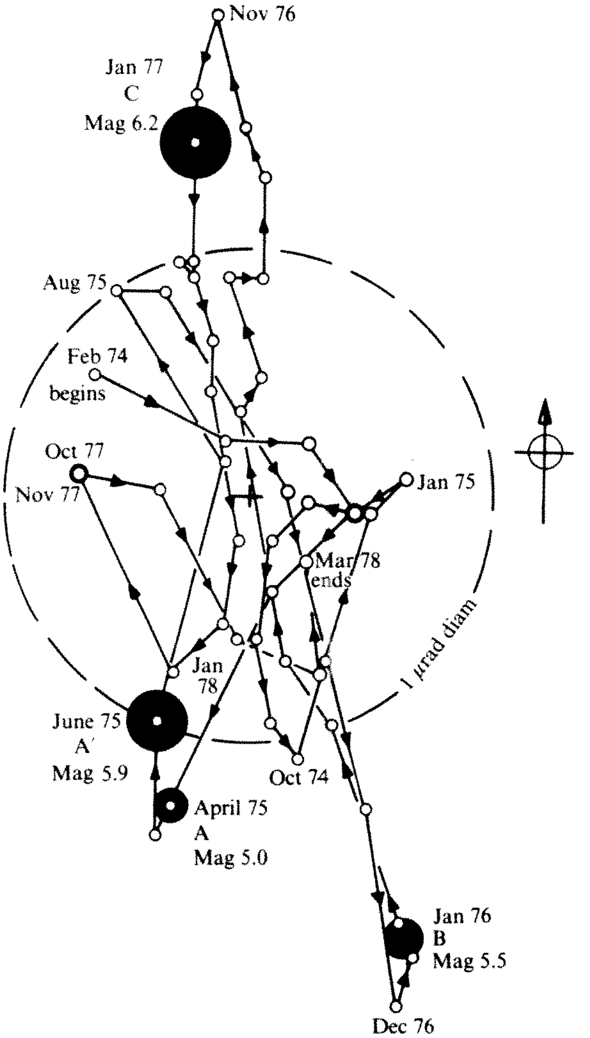

Fig. 1 Wellington Residual Earth Tilt 1974–78. Small open circles are monthly tilt values with only significant months labelled. Large solid circles are significant earthquakes lettered to correspond with Fig. 2.

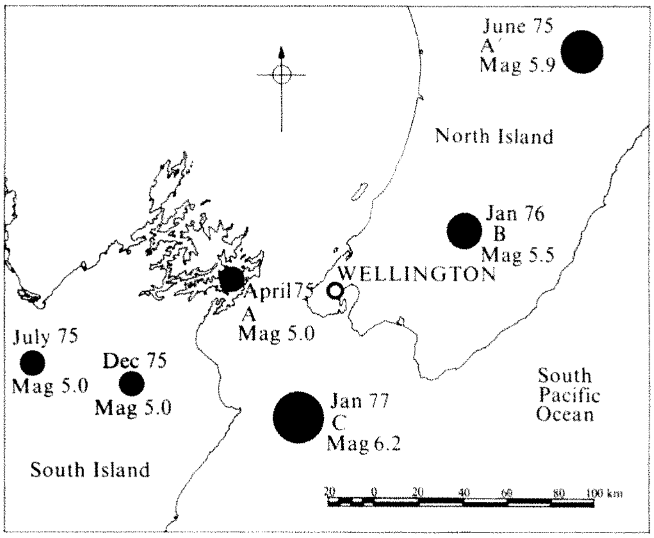

Fig. 2 Epicentres of all normal earthquakes of magnitude $M \geqslant 5$ within about 150 km of the station, during the period covered by Fig. 1. The letters refer to the corresponding earthquakes in Fig. 1.

The fit of the tilt anomalies with the two largest nearest earthquakes labelled B and C is remarkable; in each case the earthquake occurred after the tilt anomaly began to return to normal and the precursor time, while not agreeing exactly with Rikitake's[4] equation, is, nevertheless, longer for the larger earthquake even though this has the smaller anomaly. A change in the direction of tilt a month or so before the earthquake has been mentioned by Scholz *et al.*[6] in connection with Japanese earthquakes. As well as showing this effect it is also to be noted that the anomaly directions tend to be away from the future epicentres.

However, there are three possible interpretations for the first and smallest anomaly peaking in May 1975. First, it might be of no significance since it is only slightly greater than the general random walk background or it could be correlated with either earthquake A or A′. Earthquake A′ was of magnitude 5.9 and, coming in June 1975, would provide a better fit because it was then past the tilt maximum; the tilt direction is also a better fit than for earthquake A, which was smaller and nearer. Earthquake A′ was probably too far away to be detected, however. None of the other normal earthquakes were near enough or large enough to be regarded as significant. One deep earthquake, not shown, had a depth of 128 km, a magnitude of 5.9, and occurred in January 1975 having its epicentre near B. No tilt anomaly at this time is apparent and this is consistent with theories of dilatancy which suggest that a deep earthquake may not produce precursors.

Restricting the situation to normal earthquakes of $M \geqslant 5.5$ within 100 km a statistical analysis based on a Poisson distribution has yielded a probability of 3.1×10^{-3} that the overall agreement is due to chance. Thus the apparent precursory tilts are probably genuine, at least in the two clearest cases. A forthcoming damaging earthquake ($M > 6.5$, say) at a favourably situated epicentre should produce a definite detectable precursory tilt.

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Sea level–air temperature correlations near a coastal zone

A NUMBER of naturally occurring chronometric records, such as the growth rings of trees[1], distribution of pelagic fish scales preserved in anaerobic sediments[2] and the thickness of annual laminations of these sediments[3], have been correlated with time series of climatological variables. I have previously introduced sea level as one predictive variable of diatom abundance[4]; here I propose sea level as another predictive variable which can be used to estimate air temperature near the coast of San Diego (32°43′N 117°10′W) and Los Angeles (33°56′N 118°24′W). Although historical data series of sea level and air temperature have been correlated with several atmospheric and oceanic variables in the past[5], no correlation between them has, as far as I know been reported previously.

The causes of long-term (months, years) variations in sea level have been enumerated by Montgomery[6], and Lizitzin[7]. These are: (1) meteorological (atmospheric pressure, wind, precipitation and evaporation); (2) oceanographic (water density and currents); (3) river discharge; (4) the eustatic contribution; and (5) the nodal tide with the period of 18.61 yr. Factors (3) and (5) can be eliminated for the San Diego region; river discharge is negligible, and the effect of the nodal tide is expected to be very small as one of the nodal lines is located at 35°N (ref. 8), which is very close to the location under study.

Stewart *et al.*[9] investigated data recorded at several stations along the coast of North America and reported that the anomalously warm sea surface temperatures during the 1957–58 period coincided with anomalously high sea levels. Roden[10] verified this finding and reported that there was a significant correlation between monthly anomalies (deviation from long-term mean) of sea level and monthly anomalies of sea surface temperatures. Changes in sea level, during two decades, 1948–57 and 1958–69, have been studied by Namias and Huang[11]. They reported that the largest cause of variation was thermal, accounting for 3.7 cm, followed by the overall dynamic effect, 1.0 cm, and atmospheric pressure differences (the inverted barometer effect) 0.6 cm.

It is also well known that at several coastal stations of North America, including San Diego, sea surface and air temperatures are significantly correlated[12]. These findings are sufficient to hold as *a priori* that changes in sea level should be reflected in air temperatures.

Data plotted in Fig. 1 show that this is the case for both locations. Statistical methods used in obtaining Table 1 can be found in ref. 13. Briefly, average skill index calculated from N number of data points, $S_H(N)$, can be written as the sum of two terms: $S_H(N) = S_T + S_A$ where S_T is the true skill index, and S_A the artificial skill which is due to any error in sample correlations which will lead to an increase of skill above the true skill index, S_T, and depends on the number of data points: $S_A = K/N$ where K depends on time scales. The method of calculating S_H (1.0 = perfect correlation, 0.0 = no correlation) is the same as for calculating the per cent of explained variance, r^2, where r is the correlation coefficient. (For various ways of calculating S_A, see ref. 13). As can be seen in Table 1, skill indices for both locations are significantly greater than the artificial skill indices; that is, they are significantly correlated. Annual anomalies of both variables have been calculated after the effect of their respective trends have been removed.

Table 1 Trends and correlation coefficient (r), skill index, S_H (per cent of explained variance, r^2) and artificial skill, S_A

Location	Period	AT trend	SL trend	r	S_H	S_A
San Diego	1906–76	0 03 °C yr^{-1}	0.19 cm yr^{-1}	0.67	0.42	~0.04
Los Angeles	1936–75	0.04 °C yr^{-1}	−0.03 cm yr^{-1}	0.74	0.54	~0.03

Values calculated from anomalies of air temperature and sea level after both variables had been de-trended.

For statistical methods see text and ref 13. For trends and variability of yearly mean sea level calculated for various locations, see ref. 14.

It has been customary to adjust sea level data to the effect of the atmospheric pressure (theoretically, at least, sea surface should be depressed by 1 cm for every rise in atmospheric pressure of 1 mbar), however, this is questionable "since variation in atmospheric pressure is associated with change in the direction and velocity of the wind, which is one of the most important factors contributing to the fluctuations in sea level"[7]. The skill index, S_H, calculated between sea level where the 'effect' of the atmospheric pressure has been removed and air temperature at San Diego is 0.37 for the 1929–73 period, which is still higher than the artificial skill of 0.04, and is thus also predictable.

An important question is whether the use of seasonal rather than annual anomalies could improve the correlations presented in Table 1. For example, in several locations in Scandinavia cold winters are often followed by warm summers[15]; so that the annual anomalies of air temperatures might not be representative of the major changes at these locations. However, according to Namias[16], this is not true for the southern California coast. That is, negative or positive seasonal anomalies of air temperatures often persist throughout the year. The average skill index, S_H, between the seasonal anomalies of air temperature and sea level for the San Diego region during 1906–76 period is 0.29 (artificial skill, S_A, is <0.01) which is much smaller than the skill index calculated from the annual anomalies (0.42, Table 1)

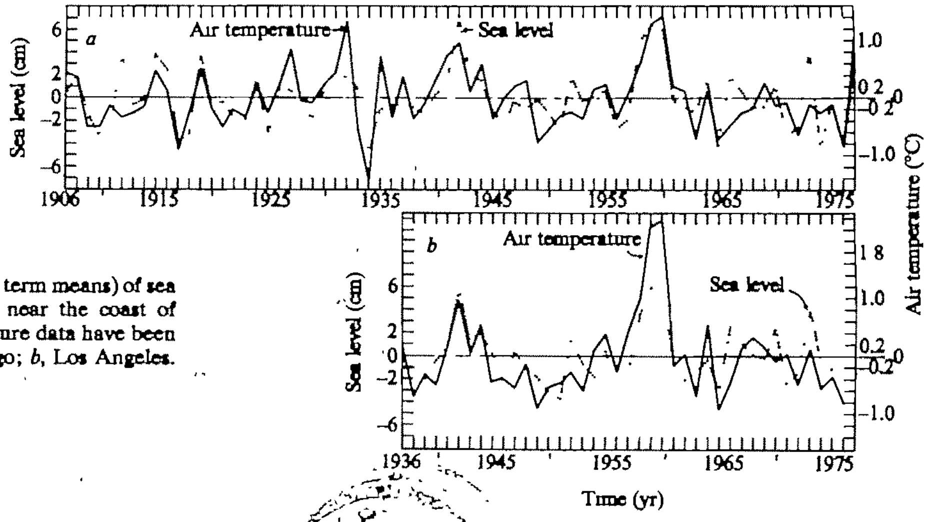

Fig. 1 Yearly anomalies (deviation from long term means) of sea level and air temperatures at two locations near the coast of southern California. Only limited air temperature data have been recorded at Los Angeles Airport. *a*, San Diego; *b*, Los Angeles.

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indicating that the use of seasonal anomalies would not improve the correlation.

At best there can only be an indirect cause-effect relationship between air temperature and sea level. However, the remarkable correlations shown in Table 1 strongly suggest that the dominant mechanisms causing fluctuations are the same. Two possible mechanisms for these fluctuations are changes in solar irradiance and wind. Previous research indicates that the most likely mechanism in this case is changes in wind systems.

Reid *et al.*[17] have reported a significant correlation between the monthly anomalies of the northerly wind component and of sea surface temperatures during spring and early summer months. Namias and Huang[11] associated the higher sea surface temperatures and higher sea levels found during 1948–57 as compared to 1958–59 period with a lesser north-to-south wind stress. After an extensive study of current, wind and sea level variations off the Oregon coast Smith[18] reported a high correlation between southwards winds and sea level. Climatological properties of the air characteristic of a location are transported when the wind deviates from its normal flow[19], thus, increased northerly winds are expected to result in lower air temperatures in this region.

This hypothesis may be an oversimplification of the complex meteorological processes which make up climate. However, from a pragmatic point of view, the existence of such a significant correlation between these two variables is of great value to the investigators of various disciplines.

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Fossil Holocene seaweed and attached calcareous polychaetes in an anoxic basin, Gulf of Mexico

DURING examination of the planktonic foraminiferal assemblages in a piston-core of black, anoxic sediments (Gyre, 77-G-13, Leg 1, 5-K) of probable early Holocene age from the Orca Basin, northern Gulf of Mexico, we discovered preserved, brown seaweed and calcareous polychaetes from a core depth of 985–1,000 cm. We know of no previous documented case of preserved seaweed in deep-sea sediments, especially with attached organisms, and we describe this material here.

The Orca Basin is a 400 km^2 depression in the continental slope of the northern Gulf of Mexico, which contains anoxic, hypersaline (about 250 g kg^{-1}) water in the bottom 200 m (ref. 1). The salt is considered to be derived by leakage from nearby diapiric structures[1]. Two piston-cores have been taken using the RV Gyre, and we have examined microfossil samples from one of these cores, which consists of black, organic-rich, fetid mud, resulting from the anoxic bottom conditions within this basin. The seaweed fragments are small (<4 mm) and the scanning electron miscroscope shows them to have clear cell walls. The core, located at 26°54′ N, 91°21.5′ W, was raised from a water depth of between 2,400 and 2,450 m and is 12 m in length. The exact depth has not yet been established because sound velocity of the brine in the basin has yet to be determined. As expected, the microfossil sequence preserved in this core is quite atypical for the Gulf of Mexico, consisting of persistent, relatively abundant, and well preserved planktonic foraminifera and radiolaria. Late Quaternary microfossil sequences outside this anoxic basin in the Gulf of Mexico differ from that of the Orca Basin by exhibiting planktonic foraminiferal assemblages that have undergone varying degrees of dissolution; pteropod assemblages occurring only spasmodically and exhibiting conspicuous dissolution and an almost complete absence of radiolarian assemblages[2]. Thus, the unusual sedimentary geochemical conditions within the Orca Basin have created an opportunity to study more complete microfossil sequences than in other areas of the Gulf of Mexico. The sequence contains persistent *Globorotalia menardii* and *Globorotalia tumida* indicating a Holocene age ($t < 10{,}000$ yr BP)[2].

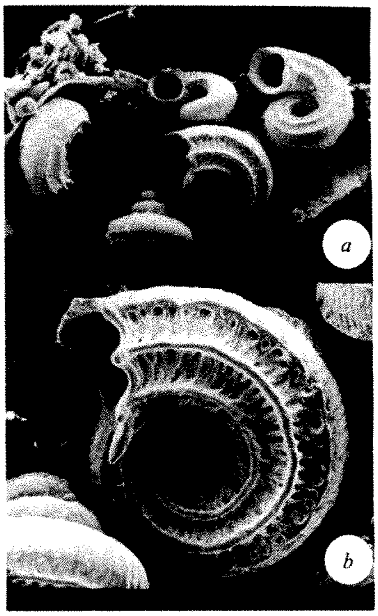

Fig. 1 *a*, Attached dextral specimen of *Janua* sp. on brown seaweed. Specimens are of early Holocene age. (Gyre Core 343, 985–1,000 cm, Orca Basin, northern Gulf of Mexico) ×26. *b*, Higher magnification view of single *Janua* sp. showing details of surface ornamentation of transverse lines and spiral ridges ×63.

We do not know of any previous documentation of seaweed in deep-sea sediments, let alone that with attached fossil organisms. Seaweed is normally very rapidly recycled within the food chain. The attached organisms (Fig. 1*a* and *b*) are calcareous, dextrally coiled polychaetes of the family Spirorbidae. The specimens have been examined (by Richard Beckwitt) and identified as *Janua* sp. based on shell and operculum characters. The specimens (Fig. 1*b*) show conspicuous transverse lines and prominent spiral ridges. The seaweed is a brown form and is probably *Sargassum* which is commonly found floating in warm waters in the Atlantic[3]. Seaweed such as this is apparently commonly deposited in the Orca Basin, as Jeffrey and Schink (personal communication) have discovered a large amount of brown seaweed in a box core from the Orca Basin. It is apparent therefore that the seaweed sank rapidly to the floor of the Orca Basin without being consumed by mid-water organisms, and was preserved in the Holocene sediments because of the anoxic conditions and a lack of benthonic organisms within the basin. Our microfossil examination of the samples revealed an almost

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complete absence of benthonic foraminifera and other benthonic microfossils, in contrast to very rich planktonic microfossil sequences (planktonic foraminifera, pteropods and radiolaria) throughout the core. We observed only about four specimens of benthonic foraminifera in the core samples examined. It is unclear whether these specimens somehow managed to live in the Orca Basin or whether they were transported in. The microfossil sequences in the core exhibit no evidence of bottom-sediment transportation. The benthonic foraminifera, however, are so rare they could have been transported in by unusual mechanisms, including seaweed transportation.

We thank Robert Tompkins and Dr David Schink for providing samples from the core. The core was collected by Robert Tompkins. We also thank Richard Beckwitt for his identification of the polychaetes. Donald Scales operated the scanning electron microscope. This research was funded by NSF grant no. OCE75-21262.

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Detection of bubbles in decompression sickness

AMATEUR diving and industrial demands have changed decompression sickness from being a specialised problem to becoming a health risk for many thousands of people. Rising medical standards and the recognition of bone damage[1] have resulted in increasingly stringent diving regulations, but the devising of suitable decompression schedules is still largely empirical; our best strategy is still to avoid sickness by accepting grave restrictions on diving procedures (such as decompression for a week or more[2]). It is important to find the earliest events involved, particularly as it is now generally accepted that the condition arises from supersaturated gas separating in the tissues; separation precedes overt symptoms and may remain symptomless[3] and experimentally at least, gas does not readily separate without some nucleus[4,5]. Methods are required applicable to man which can detect separated gas as such, and not by the physiological effect it produces, as early as possible. Various acoustic methods have been used. Bubbles moving in blood vessels have been successfully detected by the Doppler method[6,7] in experiments confirming their significance and presymptomatic existence[3]. However, this method cannot recognise stationary bubbles. We describe here a method using ultrasonic imaging that will make it possible to detect moving or static bubbles down to the required limits, in a chosen plane in the body, and hence to study at the earliest stages the location, development and movement of bubbles in a variety of tissues after subjection to decompression procedures.

The first such method used was an acoustic–optical method[8] which proved lacking in resolution. An alternative, first explored by MacKay[9], uses pulse-echo ultrasonic techniques. The method can, in principle, recognise gas at any site, static or in motion. It should therefore be possible to identify the sites of origin, and analyse the processes of bubble formation. Given this knowledge, one could hope to avoid decompression sickness without the need for tedious (and sometimes damaging) trials.

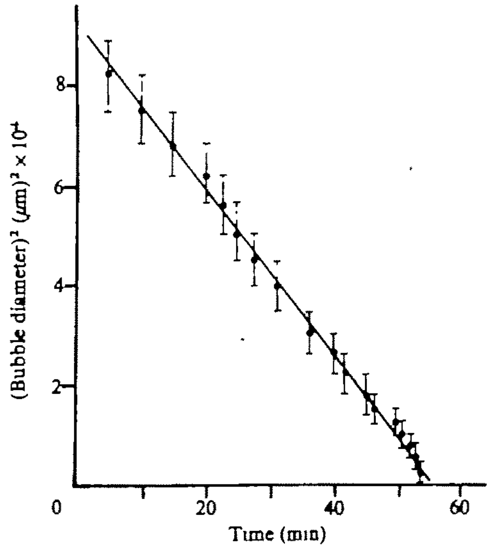
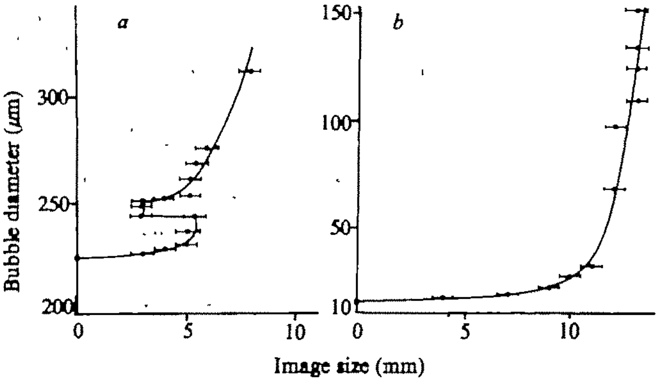


Fig. 1 Plot of bubble decay in degassed water, verifying the Epstein–Plesset equation which relates the square of bubble radius to time. A least-squares linear regression analysis gave a correlation coefficient of 0.998. The error bars represent ±10 μm in measuring bubble diameter.

Using a pulse-echo system operating at 7.5 MHz, Rubissow and MacKay[10] reported routine observation of bubbles of 2–5 μm diameter and even down to 0.5 μm in optimum conditions. Furthermore, they suggested that the actual diameter of such bubbles could be measured. The claim that such small bubbles could be detected has been challenged on theoretical grounds but without repeating the experiments[11–13]. At 7.5 MHz the wavelength of sound in tissue is 200 μm and from the relationship between target radius and back-scattered sound intensity[14] this detection limit seemed unlikely. In view of this controversy and the promise of this technique for the study of decompression sickness, an 8-MHz pulse-echo system has been developed: at this frequency, bubbles can be detected down to 10 μm diameter, measured microscopically, but not below; bubble size cannot be obtained from image size for bubbles <300 μm in diameter; and 10 μm diameter bubbles seem likely to be the smallest stable *in vivo*.

The pulse-echo system was based on two commercially available units, which after modification, provided the basic amplitude and brightness modulated displays, with sound frequency 8 MHz and pulse repetition frequency 2 kHz; using a 5-mm

Fig. 2 Ultrasonic image size as a function of gas bubble diameter, plotted by simultaneously observing the decay of the gas bubble ultrasonically and by optical microscopy, *a*, Image size measured using a low (45dB) gain. *b*, Image size measured using a high (80dB) gain. The frequency of sound used was 8 MHz corresponding to a wavelength of 187 μm (the acoustic coupling medium being water).



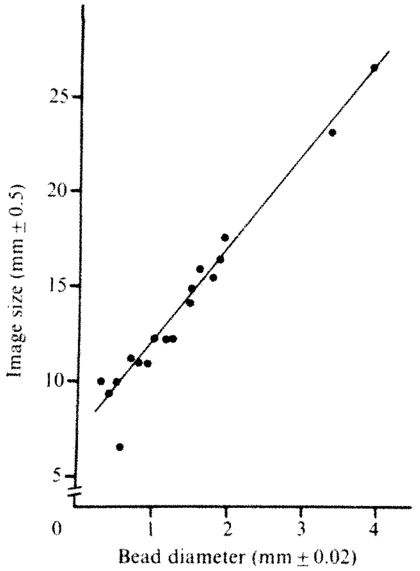


Fig. 3 Ultrasonic image size as a function of target size, measured using spherical glass targets, size range 0.4–3.5 mm diameter. The glass spheres were held in the centre of the ultrasound beam by a fine glass thread. The bead diameters were measured using a microscope fitted with a calibrated graticule. The ultrasonic image sizes were all measured from the photographic record of the images recorded from the glass beads.

diameter probe. The probe is focused with a plano-concave epoxy lens, fixed directly to the face of the PZT-5A transducer. A simple sector scan mode is used, at a frequency of 1 Hz, with the target coupled acoustically to the transducer with water. The ultrasonic images are displayed on a variable persistence oscilloscope. (Full details of the system will be published elsewhere.)

To determine the detection threshold of this system the decay of small gas bubbles in water has been observed simultaneously by the ultrasound system and by optical microscopy, which also allowed the relationship between bubble radius and image size to be determined. The test bubbles were confined in a small depression in a bubble-free block of 4% gelatin, gelatin being both acoustically and optically transparent. The block, held by a hypodermic needle, was positioned in the ultrasound beam with a micromanipulator, and coupled acoustically to the probe by degassed, distilled water. Single bubbles, of ~300 μm diameter, were formed in the cavity using a microelectrode and syringe. Microscopically such bubbles decayed over a period of several hours, and in over 24 h in air-saturated water. Their diameter could be measured to an accuracy of ±5 μm. Bubble decay followed the Epstein–Plesset equation[15], which, ignoring surface tension effects, has the approximate solution:

$$(R/R_0)^2 = 1 - (2x/R_0^2)t,$$

where R = bubble radius; t = time; and x = constant. Although the equation is expected to be inaccurate for small bubbles no appreciable deviation was found in this study (Fig. 1).

The ultrasound images of the bubbles were recorded automatically every 2 s at selected periods during their decay, and continuously when their diameter fell below 50 μm. Under the microscope, when the diameter reached 10 μm the bubbles apparently seemed to vanish suddenly, even in the presence of surfactant, detergent or when the surrounding medium was air saturated. A plot of bubble diameter against image size (Fig. 2) shows that, in optimum conditions (Fig. 2*b*), the detection limit of the ultrasound system is close to 10 μm, but there is no simple relationship (Fig. 2*a*) for bubbles with a diameter <300 μm. This result was expected from consideration of the scattering cross-sections[4,16] when the target radius approximates to the wavelength (at 8 MHz the wavelength of sound in water is 187 μm). In these conditions a variety of complex effects are expected, shown in Fig. 2*a*. Below this critical region (Fig. 2*b*) a monotonic relationship was found but its slope is either too flat or too steep for practical use. However, for bubble diameters >300 μm another simple relationship was indicated and has in fact been supported by experiments with glass spheres (0.3–4 mm diameter) as targets (Fig. 3).

These experiments show that bubbles can be detected down to 10 μm diameter and although larger than previously claimed[10] it seems to be an adequate limit, as no evidence was found microscopically for free bubbles smaller than this. Bubble size can be obtained from image size, for bubbles of diameter >300 μm, but below this a complex situation exists where an increase in bubble size may lead to a decrease in image size. One consequence is that bubble sizes cannot be calculated by applying small pressure changes and interpolating bubble size by observing the change in image size and applying Boyle's law. This technique has been used previously[10] and would lead to erroneous conclusions. Preliminary experiments have shown that the method is capable of detecting both fixed and moving gas bubbles in the legs of decompressed, anaesthetised guinea pigs.

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Somatic polymorphism and seed dispersal

SOMATIC seed polymorphism is the production of seeds of different morphologies or behaviour on different parts of the same plant and is a somatic differentiation rather than the result of genetic segregation[1]. This phenomenon appears to be confined to a limited number of families of higher plants (for example, Cruciferae, Compositae, Chenopodiaceae, Gramineae). Seed produced within a somatic polymorphism may vary in size (*Xanthium* sp.[2]), colour (*Atriplex heterosperma* Bunge[3]) and/or external structure (*Chenopodium album* L.[4]). These variations in morphology are frequently accompanied by differences in germination requirements with the consequence that the germination of polymorphic seeds may be staggered in time. Such somatic channelling of progeny into distinct classes of behaviour may increase the fitness of individuals in environments that are temporally patchy, at the expense of the intrinsic rate of increase that could be attained by a unique seed type in a

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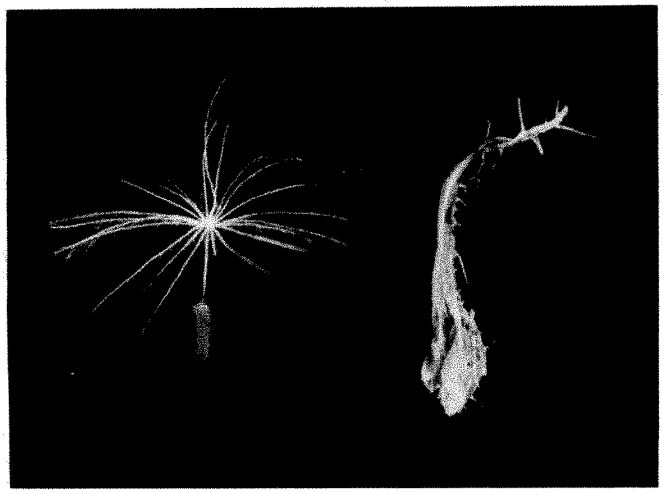
uniform environment. It might be expected that somatic seed polymorphism would also be found in species occupying habitats that are patchy in space and under these circumstances polymorphism in dispersal mechanisms could also be anticipated. One reported case may come in this category; *Gymnarrhena micracantha* Desf.[5], a desert annual, produces numerous small aerial wind-dispersed achenes (the fruit in Compositae is usually termed an achene but is strictly a pseudo-nut as the pericarp is partly axial and there is more than one carpel[6]) and also larger subterranean achenes close to the parent plant. The wind dispersed achenes are produced in greater numbers in years with high rainfall. The subterranean achenes serve only to establish descendants in the safe sites previously occupied by their parents. We report here an even more striking polymorphic dispersal mechanism in the biennial *Picris echioides* L. (Compositae).

P. echioides (bristly ox-tongue) is a fugitive species associated with woodland and wayside clearings. The plants grow to about 50 cm tall and the stems and leaves are covered with numerous bristles. Yellow flower heads are surrounded by an involucre of leafy hairy bracts. Flowers within the capitula are ligulate, the majority producing rufous achenes, transversely wrinkled towards the short beak which bears a pappus of 20 white silky hairs. However, each of the larger marginal outer achenes is tightly held in an enclosing involucral bract; these achenes are covered with short white hairs and have a shorter beak and shorter, vestigial pappus hairs (Fig. 1). Inner achenes weigh about 0.015 g and the dissected embryo weighs 0.005 g. Outer achenes weigh about 0.029 g and the embryo 0.010 g. There are approximately 25 inner and six outer achenes per involucre. The inner achenes are clearly wind dispersed, although the pappus and beak readily break off during or after dispersal. The outer achenes remain within their bracts when the inflorescence falls as a whole. The bracts are hairy and sharply bent, suggestive of animal dispersed propagules.

Three hundred pappus-bearing inner and 300 bract-enclosed outer achenes were scattered randomly on the floor of a 90 cm × 60 cm plastic enclosure. Two laboratory mice (*Mus musculus*) were released in the container for 35 min; the number of seeds transported, the seed retention time and the behaviour of the mice were recorded. In a further experiment a laboratory mouse was placed in a straw-lined 120 cm × 60 cm enclosure, fed on a diet of laboratory pellets and seeds of *Festuca rubra* and allowed to habituate for 7 d, during which time the mouse established a nest. Two hundred and fifty inner achenes and 250 outer achenes (in bracts on senesced flowerheads) were scattered randomly within the enclosure. The transport of achenes to the nest by the mouse was recorded daily. The outer achenes stuck to the forefeet, hindfeet and tail base of the mice in the first experiment; several outer achenes were carried for up to 195 s (Fig. 2). The adhered achenes did not appear to irritate the mice, nor did they seem to be attractive as a food source. In a few cases the mice actively removed the seeds by chewing but the majority of achenes dropped off passively. No inner achenes were ever observed to adhere to a mouse. A daily count of seeds in the second experiment showed that there was a continued transport of senesced flowerheads (containing outer achenes) to the established nest of the mouse. Again no inner achenes were ever transported in this manner.

Fig. 1 Achenes of *Picris echioides*. Left, inner pappus-bearing achene; right, outer achene enclosed in involucral bract.



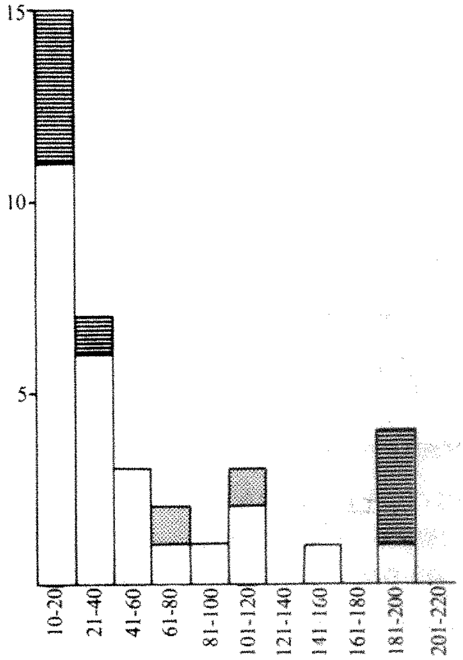


Fig. 2 Frequency distribution of the time for which the outer (bract-enclosed) achenes of *Picris echioides* remained attached to laboratory mice. No inner (pappus-bearing) achenes were ever observed to adhere to the mice. ▤, Attached to tail; □, attached to forefoot; ▩, attached to hindfoot.

The transport of whole capitula implied active caching or the removal of attached flowerheads through grooming at the nest site. The disinterest of mice in outer achenes as a food source in the first experiment and the high chaff to embryo ratio of outer achenes, which must reduce their digestive value, suggests that caching is unlikely. Instead the accumulation of outer achenes in the nest appears to be due to passive transport followed by grooming.

It might be expected that the two types of achene, which differ in size, would have different dormancies, rates of seedling growth or competitive ability (particularly when grown in mixed populations[7]). A series of experiments revealed no such differences[8]. It appears therefore that the achene dimorphism in *Picris echioides* may be related simply to differences in dispersal behaviour. In nature, potential animal dispersers of *P. echioides* include *Clethrionomys glareolus*, *Microtus agrestis*, *Micromys minicticus* and *Apodemus sylvaticus*. The behavioural patterns and nesting habits of these small mammals may well effect transport of the seeds of *P. echioides* into habitats quite different from those that would be reached by wind dispersal.

Somatic seed polymorphism results in the production of a constant ratio of seed types regardless of short term 'patchiness' of the environment and prevents 'tracking' of transient changes. This phenomenon contrasts with the genetic polymorphism of seed dispersal mechanisms such as that found in *Aegilops speltoides* Tausch[9] in which two kinds of dispersal unit are borne, but on different individuals. Such genetic polymorphism is sensitive to changing direction and intensities of selection, hence the observed fixation of monomorphic populations on the fringes of the distribution area of *A. speltoides*. In contrast, somatic polymorphisms as in *P. echioides*, are buffered against rapid response to selection; all individual plants are monomorphic and every individual appears to bear polymorphic achenes.

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Cell sorter analysis of leukaemia-associated antigens on human myeloid precursors

NORMAL and malignant B cells, some monocytes and blast cells from both the common (non-T, non-B) form of acute lymphoblastic leukaemia (ALL) and acute myeloblastic leukaemia (AML) share Ia-like antigenic determinants[1-4]. ALL cells, but not AML cells, can be further characterised by the expression of an 'ALL-associated' membrane antigen[9,10]. Although the ALL antigen and the Ia-like antigens on leukaemic blasts have at one time been regarded as candidate leukaemia-specific antigens, it has been suggested that both may be normal differentiation antigens of haematopoietic precursors, their expression in leukaemia reflecting the cellular origins of the disease[2,7-11]. In immunofluorescent studies combined with phase contrast examination, Ia-like antigens can be demonstrated on normal and leukaemic myeloblasts and some promyelocytes, but not on most promyelocytes and maturing granulocytic or erythroid cells[7,8]. The presence of Ia-like antigens on myeloid-committed stem cells is supported indirectly by results of functional assays *in vitro* which show the abolition of myeloid colony-forming cells (CFUc) by incubation with complement and hetero-antisera to B cells[12,13]. Two disadvantages of the approach used in the latter experiments are, first, that the cells of interest are eliminated and no longer available for analysis, and second, that their functional inhibition does not necessarily establish that they carry the antigens in question, as the development of colonies involves stimulatory factors[14,15] and auxiliary cells, whose activity, rather than CFUc *per se*, might be compromised by the antibodies used. We now report direct evidence for the presence of the antigens on bone marrow cells by separating them in a fluorescence-activated cell sorter (FACS), according to their binding of anti-Ia-like (anti-p28,33) or anti-ALL antibodies. Functional analysis of the resultant populations reveals that a proportion of human colony and cluster forming cells express the Ia-like hetero-antigen but not the ALL-associated antigen.

Table 2 Morphology of separated Ia$^+$ and Ia$^-$ bone marrow cells

	Adult bone marrow Unsorted	Adult bone marrow Ia$^+$	Adult bone marrow Ia$^-$	Fetal bone marrow Unsorted	Fetal bone marrow Ia$^+$	Fetal bone marrow Ia$^-$
Blast cells	2.5	35.5	2	13	33*	8
Promyelocytes	1	32.5	2	7	10	8
Myelocytes	30	12.5	42	17	2	18
Maturing granulocytic cells	29.5	6	21	11	3	30
Monocytes	7.5	3	3	3	3	4
Red cell precursors	16	2	20	9	0	10
Lymphocytes	13	7.5	10	31	36	14

Cells were prepared as described in Table 1 and separated on FACS. Smears were made and stained with May–Grünwald–Giemsa. Differential counts are shown as percentages.

*Most cells are myeloblasts. Some resemble lymphoblasts.

For convenience, we refer below to the anti-p28,33 sera as anti-Ia and to the cells bearing the p28,33 determinants as Ia$^+$. This terminology presumes more than is proven of the genes involved in the control of these molecules in man; however, the human B cell p28,33 complex has strong structural similarities to rodent Ia molecules[4,16,17]. The 33K component in man is apparently regulated by genes on chromosome 6 (ref. 18) and carries the polymorphic determinants which are identified by human anti-(HLA) *D* locus sera[19,20]. The membrane antigens specifically reactive with anti-p28,33 on leukaemic blasts are, as on B cells, associated with a noncovalently bonded p28,33 complex[21]. The anti-ALL antibodies used here, although produced by immunisation with whole cells, selectively bind to a single glycosylated polypeptide species with an apparent molecular weight of 100,000 (ref. 22). Reactive cells are referred to as ALL$^+$.

After elimination of most mature granulocytic and erythroid cells by Ficoll–Triosil density centrifugation, adult marrow samples contained 10–14% cells reactive with anti-Ia serum (Table 1). Ia$^+$ cells included larger cells, mainly myeloblasts

Table 1 Characterisation of Ia$^+$ cells in human bone marrow with membrane markers

Source of bone marrow	Adult (61 yr)			Fetus (19 weeks)			Regenerating (6 yr) (off treatment for ALL)		
	Total*	Small*	Large*	Total	Small	Large	Total	Small	Large
Cells reacting with:									
Normal rabbit serum	<1			<1			<1		
Anti-Ia-like serum (Ia$^+$)	14	4	10	48	30	18	32	20	12
Anti-ALL serum (ALL)$^+$	<1			13	12†	1	14	14†	0
Anti-Ig (SmIg$^+$)	5	4	1	10	10†	0	10	9†	1

To eliminate red cells, normoblasts and granulocytes, cells were separated on Ficoll–Triosil and treated with 0.17 M NH_4Cl for 15 min. The cells were then passed through fetal calf serum and washed The antisera used were ultracentrifuged before use (100,000*g* for 1 h). Cells were incubated for 20 min at 4 °C with either normal rabbit serum (NRS 1 10 dilution) or rabbit anti–Ia serum made against purified human p28,33 Ia-like antigens (Ia; 1:20 dilution)[4,5,8] or rabbit anti-ALL serum (ALL; 1:10 dilution)[9,21], washed three times, incubated for 20 min with fluoresceinated goat anti-rabbit immunoglobulin (1:50 dilution) and washed three times again Other samples were incubated with goat anti-human immunoglobulin directly labelled with fluorescein or rhodamine-isothiocyanate (anti-Ig-FITC or anti-Ig-TRITC))1:10 dilution) and washed three times The samples shown were analysed by the FACS (see Fig. 1). Adult bone marrow samples were obtained from ribs removed during operation for lung cancer. Patients were 55–65 yr old. Fetal bone marrow samples were from 19–21-week-old fetuses. Representative experiments are shown. Regenerating marrow samples were obtained from children after the completion of chemotherapy regimes given for the treatment of acute lymphoblastic leukaemia. The bone marrow sample shown was taken from a 6-yr-old child who had been off treatment for 8 months. The marrow showed moderate lymphocytosis. The analysis was carried out 7 months ago and the child is still in complete remission of his leukaemia.

*% of cells showing positive membrane staining under UV microscope

†Double marker experiments (anti-ALL: FITC; anti-Ig: TRITC) showed that ALL$^+$ cells were SmIg$^-$, and SmIg$^+$ cells were ALL$^-$ (ref. 11). Ia$^+$ lymphocytes failed to form rosettes with sheep erythrocytes and did not react with anti-thymocyte serum[22].

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Table 3 Characterisation of Ia$^+$ cells producing granulocyte–macrophage clones *in vitro*

Source of bone marrow	Adult		Fetus		Regenerating (off ALL treatment)	
	a	*b*	*a*	*b*	*a*	*b*
Cells were incubated with:						
1 None	38.1 ± 7.5		52.5 ± 4.0		211.1 ± 22.2	
2 NRS	32.0 ± 14.3		NT		248.9 ± 15.6*	
3*a* Anti-Ia serum, unsorted	61.2 ± 6.2		73.1 ± 6.1		272.4 ± 9.3	
3*b* Sorted Ia$^+$ cells, total	110.7 ± 15.2	15.5(47.3%)	196.1 ± 30.2	94.1(90.5%)	—	
3*c* Sorted Ia$^+$ cells, small	NT		2.3 ± 1.6		56.0 ± 17.8	11.2(3.2%)
3*d* Sorted Ia$^+$ cells, large	NT		153.7 ± 11.4		456.4 ± 102.2	54.8(15.8%)
3*e* Sorted Ia$^-$ cells	20.1 ± 5.7	17.3(52.7%)	19.1 ± 0.8	9.9(9.5%)	413.3 ± 81.4	281.0(81.0%)
Calculated totals after sorting		32.8(100%)		104.0(100%)		347.0(100%)

Preparation of cell suspensions is shown in Table 1. Histograms of the stained cell suspensions were made on the FACS (Fig. 1) and Ia$^+$ large and small cells and Ia$^-$ cells were separated. Cells in each fraction were counted, plated in double-layer agar cultures[15] and incubated for 7 d. In all experiments, changes in numbers of colonies (>40 cells per clone) corresponded with changes in clusters (<40 cells per clone). In addition, no difference was detected in the proportion of compact (granulocytic) and diffuse (macrophages + mixed) colonies. NT, not tested. *a*, Observed values expressed as numbers (±s.d.) of colonies and clusters (that is, clone-forming cells) per 10^4 cells plated. *b*, Calculated numbers of Ia$^+$ and Ia$^-$ clone-forming cells in 10^4 cells of the original cell population. The formula is: value *a* × proportion of Ia$^+$ or Ia$^-$ cells (from Table 1). The sum of Ia$^+$ and Ia$^-$ *b* values shows the total number of clone-forming cells per 10^4 cells recovered after sorting. If this is taken as an arbitrary 100%, then the relative contribution of Ia$^+$ and Ia$^-$ cells can be given (in parenthesis).

*These cells were passed through the FACS but were not sorted.

and promyelocytes (Table 2), and smaller SmIg$^+$ (surface membrane-immunoglobulin-positive) B lymphocytes. The Ia$^-$ cells were myelocytes, maturing granulocytic and erythroid cells, a few lymphocytes and <5% myeloblasts and promyelocytes.

In normal fetal bone marrow and regenerating juvenile marrow, more cells (30–50%) reacted with anti-Ia serum than in adult marrow (Fig. 1*b,c*, Table 1). Again, the Ia$^+$ cells included large lymphoblasts, myeloblasts and promyelocytes as well as small lymphocytes. The latter were heterogeneous by membrane marker criteria: 8–15% were B lymphocytes (SmIg$^+$, ALL$^-$) and the remaining lymphocytes reacted weakly with anti-ALL serum (ALL$^+$), were negative for SmIg (SmIg$^-$) and failed to form rosettes with sheep erythrocytes or to react with anti-thymocyte serum. These non-T,non-B ALL$^+$ cells are not readily detectable in the adult marrow samples[9,11]. As in the adult marrow, the Ia$^-$ cells in fetal and regenerating marrows were mainly maturing granulocytic and erythroid cells; however, Ia$^-$ myeloblasts were also seen (Table 2).

The data obtained in the myeloid clonal assays are shown in Table 3. The binding of anti-Ia antibodies to bone marrow cells in the absence of complement (Table 3, line 3*a*) and manipulations with the cells on the FACS (Table 3, line 2, last column) neither inhibited nor significantly stimulated clone-forming cells. A striking finding was that colony and cluster-forming cells were present in both Ia$^+$ and Ia$^-$ fractions. Accumulation of these clone-forming cells in the Ia$^+$ fraction (Table 3, line 3*b*) with concomitant depletion in the Ia$^-$ fraction (Table 3, line 3*e*) was observed in five of seven experiments. Note, however, that in the Ia$^+$ fractions invariably fewer cells were observed than in the Ia$^-$ fractions (Table 1). When the total contribution of the different cell populations to the colony and cluster-forming cells was calculated in the different experiments, the Ia$^+$ cells contained 10–80% of clone-forming cells (mean 40%, seven experiments). Representative values are shown in Table 3. The remaining clone-forming cells (mean 60%) were sorted into the Ia$^-$ fractions. The wide diversity of Ia expression on clone-forming cells was confirmed when adult marrow cells were separated into strongly Ia$^+$, moderately Ia$^+$ and Ia$^-$ fractions. Colony and cluster-forming cells were observed in all three populations (Table 4). Mixed and pure neutrophil–granulocyte and macrophage clones and pure eosinophil clusters (8–14%) were found in cultures of all fractions.

Clone-forming cells were found almost exclusively among the intermediate-to-large cells (Table 3, line 3*d*), suggesting that small Ia$^+$, ALL$^+$ non-T,non-B cells did not form myeloid colonies. This notion was directly confirmed. Fetal marrow cells were stained with anti-ALL serum, separated on the FACS and cultured for 7 d. The ALL$^+$ and ALL$^-$ fractions gave, respectively, 3.2 and 626 clones per 10^4 cells.

We interpret these results to suggest that a proportion of human myeloid precursors are Ia$^+$ and a few of them are very

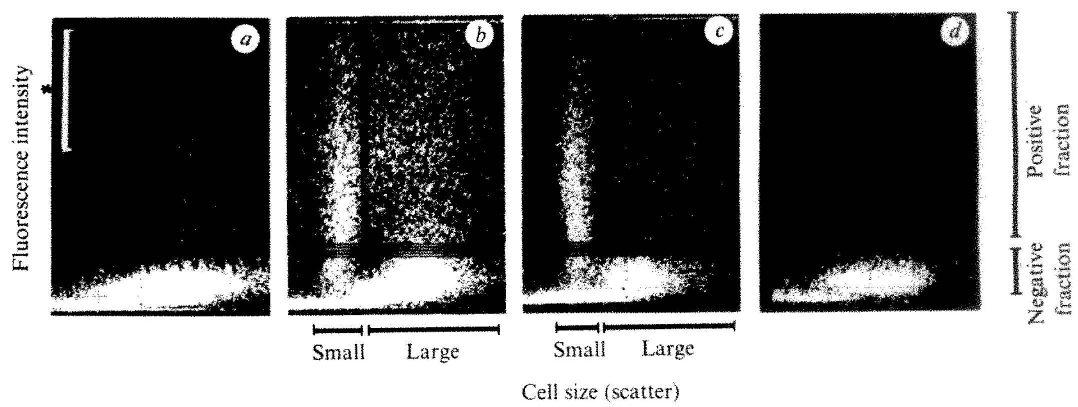

Fig. 1 Bone marrow cells were stained with anti-Ia serum (*a–c*) or normal rabbit serum (*d*, negative control) as described in Table 1. Histograms made on the FACS-1 (Becton Dickinson) are shown. The cells were separated on the basis of fluorescence intensity (Ia$^+$ compared with Ia$^-$ fractions). Ia positivity was arbitrarily defined as staining intensity above negative controls (*d*). The nonspecific staining in suspensions incubated with NRS was checked on the UV microscope in each experiment and found to be minimal. Some samples were also separated on the basis of cell size (small against large) as indicated. The cells within the shaded areas were discarded. The standard conditions of FACS setting were: laser power 400 mW at 488 nm; scatter channel gain 2; fluorescence channel gain 8; photomultiplier tube 700 V (see footnote to Table 4). In these conditions the proportions of Ia$^+$ and Ia$^-$ cells detected on the FACS agreed with the observations made on the UV microscope (Table 1). *a*, Adult; *b*, fetal; *c*, regenerating.

Table 4 Colony and cluster-forming cells express a wide range of Ia densities on the cell membrane

Strong Ia$^+$	581 ± 172*
Moderately Ia$^+$	449 ± 24
Ia$^-$	217 ± 44
Unseparated	277 ± 57

Normal adult bone marrow cells were stained with anti-Ia serum and separated into strongly Ia$^+$, moderately Ia$^+$ and Ia$^-$ fractions on the FACS and cultured (clones per 10^4 cells plated ± s.d.).

*Intensity of fluorescent staining is shown in Fig. 1*a* by an asterisk.

strongly Ia$^+$. As very weakly Ia$^+$ cells might have been sorted into the Ia$^-$ fraction in the conditions used on the FACS, our results do not exclude that a larger proportion of clone-forming cells carry Ia-like antigens when analysed by other techniques, for example, by complement-mediated cytolysis. Nevertheless, our data indicate a striking heterogeneity of Ia expression on myeloid precursors which could not be appropriately documented with other techniques. The results also strongly suggest that small cells of lymphoid morphology with the antigenic phenotype of acute lymphoblastic leukaemia (ALL$^+$, Ia$^+$) are not CFUc. This fact has not been unequivocally established, as ALL$^+$ cells might require the collaboration of large Ia$^+$ cells for their development into colonies. This possibility seems unlikely, however, as in our experiments the separated cells were cultured on peripheral blood feeder layers which provided an ample source of colony-stimulating factors[23].

These findings suggest that the two distinct Ia$^+$ non-T,non-B cell types (that is, the smaller ALL$^+$ cell and the larger ALL$^-$ cell which express some myeloid characteristics) might represent the target cells for leukaemic transformation in the common (non-T,non-B) form of ALL and in AML respectively. This implies that leukaemic blasts largely retain at least some of the antigenic characteristics of cells from which they are derived[11]. Alternatively, the target cell in ALL and AML could be an even less mature haematopoietic stem cell, subsequent maturation of the leukaemic clones being 'frozen' at the level corresponding to the two cell types. The nature of the ALL$^+$, Ia$^+$ 'lymphoid' cells is unknown; we regard these cells as candidate lymphocyte precursors or stem cells[11].

These and previous studies[7,8,24,25] indicate a maturation-linked expression of Ia-like antigens with a progressive loss during granulocyte differentiation. Whether the striking variability of Ia-like antigen expression on CFUc cells is related to particular stages of cell differentiation, governed by events linked to the DNA synthetic cell cycle[25,26] or due to other factors is essentially unknown and warrants further study.

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Novel form of T-cell stimulation of immunoglobulin secretion

THE precise mechanism of action by which helper T cells induce precursor B cells to acquire the capacity to secrete immunoglobulins[1,2] remains poorly understood. This paucity of information is due, in part, to the temporal lag between the initial T cell–B cell interactions relatively early in the process and the subsequent expression of these interactions a day or more later[3,4]. This time lag presumably relates to the time required for B cells to undergo a maturation or differentiation after interaction with T cells. We now report that, using a pulse-label technique, we have observed that the addition of thymus cells to previously cultured spleen populations during the 90-min pulse-label period resulted in a marked increase in the rate of secretion of immunoglobulin. Moreover, the enhancement occurred without detectable delay.

Unimmunised New Zealand white rabbits were used as a source of lymphoid tissue. The method for preparing cell

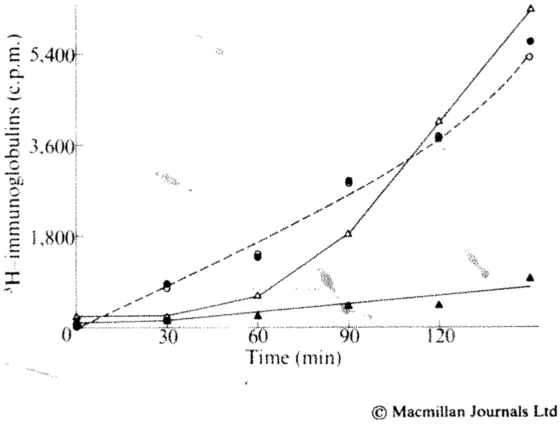

Fig. 1 Effect of thymocytes on the rate of appearance of intracellular and extracellular ^{3}H-immunoglobulin. The procedure is described in the legend to Table 1. Spleen cells were cultured for 72 h before use and thymocytes were isolated on the day of use. ▲, △, Extracellular fluid; ●, ○, intracellular fluid. Open symbols, both splenocytes and thymocytes; closed symbols, splenocytes only.

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suspensions[5] and the procedure used for tissue culture were as previously described[6,7]. Assessment of immunoglobulin production by coprecipitation analysis[8] and the preparation and specificity characteristics of anti-thymocyte serum has also been described[7,9]. Spleen cells were centrifuged on a Ficoll gradient and those cells concentrated atop the 30% layer were used in cell culture[10].

The effect of mixing separately cultured splenocytes and thymocytes is shown in Table 1. Although freshly isolated spleen cells failed to exhibit increased ^{3}H-immunoglobulin secretion on addition of thymocytes (experiment 1), spleen and thymus cells, cultured separately for 2 or 3 days before being mixed at the beginning of the 90-min pulse-label period, yielded increased ^{3}H-immunoglobulin secretion above that observed with spleen alone.

From the results of experiment 1 it was not clear whether both thymus and spleen cells required a prior culture period before being mixed in the pulse-label assay. Accordingly, an experiment was performed in which the duration of prior incubation for each cell type was varied. It can be seen (Table 1, experiment 2) that it was necessary to culture spleen cells for at least 2 days and preferably for 3 days in order to observe the rapid onset effect of thymus cells. In contrast, prior incubation of thymus cells was not required and, indeed, tended to reduce the extent of stimulation that was ordinarily observed with fresh thymus cells.

Table 1 Effect of mixing separately cultured splenocytes and thymocytes

Expt no.	Cells present during pulse-label period	Length of prior incubation of: spleen	thymus	^{3}H-immunoglobulin secreted during pulse-label period
		(d)	(d)	(c.p.m.)
1	Spleen	0	NA*	60
	Spleen + thymus	0	0	40
	Spleen	1	NA	90
	Spleen + thymus	1	1	80
	Spleen	2	NA	360
	Spleen + thymus	2	2	680
	Spleen	3	NA	720
	Spleen + thymus	3	3	2,570
2	Spleen	3	NA	230
	Spleen + thymus	3	3	1,530
	Spleen + thymus	3	2	3,450
	Spleen + thymus	3	1	3,210
	Spleen + thymus	3	0	4.970
	Spleen	2	NA	90
	Spleen + thymus	2	3	200
	Spleen + thymus	2	2	360
	Spleen + thymus	2	1	510
	Spleen + thymus	2	0	750
	Spleen	1	NA	50
	Spleen + thymus	1	3	60
	Spleen + thymus	1	2	80
	Spleen + thymus	1	1	70
	Spleen + thymus	1	0	100

Spleen and thymus cells were separately cultured in Medium 199 containing 10% fetal calf serum for the indicated number of days. After being washed with leucine-free medium 199, spleen cells (10^{7}) with or without thymus cells (6×10^{7}) were incubated in the presence of such medium containing ^{3}H-leucine for 90 min at 37 °C. At the end of this period, the ^{3}H-immunoglobulins secreted into the extracellular fluids were assessed by coprecipitation of one aliquot in the presence of carrier rabbit immunoglobulin and goat anti-rabbit immunoglobulin and another aliquot in the presence of egg albumin and goat anti-egg albumin. The difference in these samples reflects specifically precipitable rabbit immunoglobulins. The values shown for mixtures of spleen and thymus have been corrected for the small amounts of ^{3}H-immunoglobulin detected when thymus cells were incubated alone. This correction corresponded to 1% of the ^{3}H-immunoglobulins found when spleen and thymus cells were incubated together.

* NA, not applicable.

Table 2 Evidence for T-cell participation in enhancement of secretion of immunoglobulin

Cells added to spleen cells	^{3}H-immunoglobulin secreted
	(c.p.m.)
None	530
Thymus	2,200
Thymus (ATS*-treated)	740
None	400
T-cells (nylon wool)	2,300
Thymus	2,600

The procedure is described in the legend to Table 1. Spleen cells were cultured for 72 h before use, whereas the thymus cells used were freshly isolated. Spleen T-cell enriched populations were prepared on nylon wool columns[13] using freshly isolated cells.

* ATS, antithymocyte serum.

Analysis of the effect of thymocytes on the rate of appearance of intra- and extracellular ^{3}H-immunoglobulins revealed that the rate of appearance of intracellular ^{3}H-immunoglobulins within spleen cells was essentially unaltered by the presence of thymus cells, whereas thymocytes markedly enhanced the rate of appearance of extracellular immunoglobulin (Fig. 1). However, meaningful comparison of the rate of appearance of intracellular and extracellular immunoglobulins may not be possible because intracellular ^{3}H-immunoglobulins are composed not only of molecules destined for secretion but also of molecules that are sequestered, for example, surface immunoglobulins. Furthermore, since the proportion of cells containing intracellular ^{3}H-immunoglobulins which respond to thymus cells is not known, the curves for the intra- and extracellular appearance of ^{3}H-immunoglobulins may not be directly related.

In other experiments using shorter time intervals, the initial thymocyte-induced enhancement of ^{3}H-immunoglobulin secretion was detectable at 20 min (data not shown). The precise time of onset of enhanced ^{3}H-immunoglobulin secretion due to thymocytes cannot be closely defined for a period earlier than 15–20 min because lymphoid cells exhibit a secretory lag of approximately 15 min[11,12] during which little or none of the readily detectable intracellular ^{3}H-immunoglobulins can be observed extracellularly. Considered in this context there was essentially no delay in the onset of enhanced secretion due to thymus cells.

The effect of thymocytes was specific in that the rate of extracellular appearance of ^{3}H-labelled serum albumin from liver cells was not enhanced when liver cells were incubated in the presence of thymus cells. Evidence consistent with the view that T cells participate in the rapid onset enhancement of immunoglobulin secretion is shown in Table 2. Thus, the stimulation by thymocytes was lost by prior treatment with antithymocyte serum and complement. In addition, freshly isolated splenic T-cell enriched populations[13] were as efficacious as thymocytes.

Of the ^{3}H-immunoglobulins secreted in response to thymus cells approximately 65% were accountable for as IgM and 35% as IgA by specific coprecipitation analysis. In contrast, ^{3}H-immunoglobulins of the IgG class were not detected. The foregoing experiments have been performed using spleen cells cultured in the absence of added antigen and the resulting immunoglobulin secretion measured by a pulse-label assay. Recently, we have been able to detect the same rapid onset enhancement due to thymus cells using spleen cells cultured with sheep red blood cells (RBC) as antigen and a plaque assay for sheep RBC.

It will be of considerable interest to determine whether the secretagogue-like activity of thymocytes induces a substantial change in B cells or a subtle change. For example, does the alteration require the assembly or reinforcement of most of the cells' total secretory apparatus or does it simply alter the rate of release of completed molecules awaiting discharge at some terminal compartment very close to the end of the intracellular translocation process?

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Absence of H–Y antigen in XY females with dysgenetic gonads

WITH a few exceptions[1–6], the mammalian testis is known to differentiate in the presence of a Y chromosome[7], but the mechanism(s) involved remains unknown. Increasing experimental evidence shows that the testis-determining gene product is a plasma membrane protein known as H–Y antigen[8]. Several investigators have demonstrated that the gene(s) controlling H–Y antigen expression are located on the Y chromosome[9,10]. We now report the absence of H–Y antigen in 46 XY pure gonadal dysgenesis (PGD) in which patients have female internal and external genitalia and 'streak' gonads.

We studied nine subjects (20–38 yr old), three normal males, three normal females and three phenotypic females with primary amenorrhoea. Before treatment the three patients had the limb proportions of eunuchs, and had no breast development or axillary hair, although a few coarse hairs were present on the labia majora. There were no signs of Turner's syndrome. These three patients could therefore be categorised under PGD. Two of them were siblings.

Cytological studies using fluorescent and G-banding techniques on all the three patients confirmed their 46 XY karyotype (Table 1). H–Y antigen was assayed immunologically by the cytotoxicity test[11] (Fig. 1). We found that cells from PGD, like those from normal females, did not express H–Y antigen.

There are reports of the familial occurrence of this condition consistent with X-linked inheritance[12]. The locus responsible could be the same as that involved in the XY female wood lemmings[13].

Table 1 Cytological status of nine subjects

Diagnosis	Age range (yr)	X chromatin	Y chromatin	Karyotype from blood	No. of metaphase plates scored
Normal male					
($n = 3$)	25–35	–	+	46 XY	75
Normal female					
($n = 3$)	25–38	+	–	46 XX	65
PGD					
S.M.	28	–	+	46 XY	70
N.B.	36	–	+	46 XY	75
P.B.	20	–	+	46 XY	70

N.B. and P.B. are sibs.

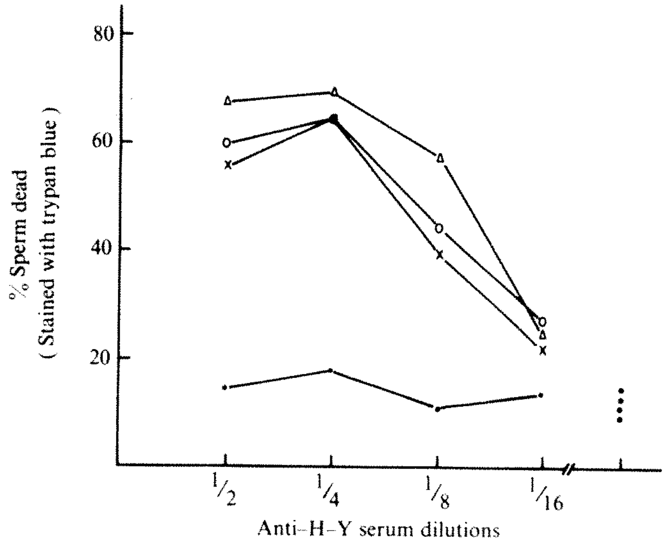

Fig. 1 Cytotoxicity test on mouse sperm with mouse H–Y antiserum after absorption with lymphocytes from three patients with PGD, three normal males and three normal females. Control (C) includes complement but no H–Y antiserum. Each point represents an average value for three separate tests. H–Y antisera were raised in C57 BL/6 female mice by five weekly intraperitoneal injections of spleen cells (4.5×10^6) from C57 BL/6 male mice. Absorption was accomplished by suspending a fixed number of lymphocytes (10^7 cells ml^{-1}) in small tubes containing 0.15 ml of H–Y antiserum diluted one half, and by leaving the suspension for 40 min on ice. Suspensions were agitated gently every 10 min. After absorption, the tubes were centrifuged into aliquots for use in the cytotoxicity test. Cytotoxicity was demonstrated with C57 BL/6 epididymal sperm according to the method of Goldberg *et al.*[11]. Equal volumes (0.05 ml) of H–Y antiserum (serially diluted 1/2 to 1/16), sperm (5×10^6 ml^{-1}) and absorbed rabbit serum (complement source diluted 1/4) were incubated at 37 °C for 50 min. Trypan blue dye (0.1 ml) was added during the last 10 min of incubation to stain dead sperm, and cells were counted in a haemacytometer. The failure of loss of cytotoxicity after absorption with female and PGD cells indicates that these cells do not contain H–T antigen. △, Unabsorbed antiserum; ○, absorbed, female; ×, absorbed, PGD; ●, absorbed, male.

There are three possible explanations of our observations as well as those from XY female wood lemmings: (1) inhibition of the testis-determining gene on the Y chromosome by abnormal gene(s) carried either on the X chromosome or on an autosome(s)[14]; (2) a mutation at the H–Y locus of the Y chromosome probably inactivating the gene product so that its essential function(s) is affected; (3) an X-linked Y suppressor gene that blocks the expression of the H–Y antigen[15]. Whatever the mechanism, it is certain that in the absence of H–Y antigen, as in our cases of PGD, the gonad could not be differentiated into testis. Since Mullerian duct derivatives such as uterus, fallopian tubes and vagina are present in all our patients, it is highly probable that blocking of the expression of H–Y antigen has occurred before initiation of gonadal differentiation.

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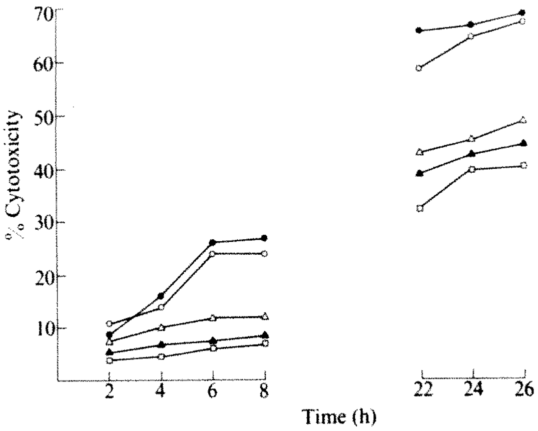
H-2 restriction and non-restriction of T-cell-mediated cytotoxicity against mouse mammary tumour targets

CELL-MEDIATED CYTOTOXICITY (CMC) against C3H/Umc (C3H) syngeneic mammary tumours measured *in vitro*, after *in vivo* immunisation, is mediated by cytotoxic T lymphocytes (CTL) and is directed mostly against cross-reacting antigens related to the mouse mammary tumour virus (MTV)$^{1-3}$. The long-term CMC assay required for detecting this response against adherent target cells has complex kinetics and at least two superimposing components: (1) an initial early peak of cytotoxicity, detectable at approximately 6 h and accounting for 20–30% of the CMC, and (2) a later peak, detectable after 18–20 h and representing an additional 40–50% of the cytotoxic effect^3. Both peaks are mediated by CTL with the Ly23 phenotype (that is, Ly1$^-$Ly2,3$^+$); however, non-cytotoxic T cells of Ly1 and probably Ly123 phenotype (that is, Ly1$^+$Ly2,3$^-$ and Ly1$^+$Ly2,3$^+$, see refs 3 and 4 for nomenclature and functional characteristics of these T-cell subsets) are required for the expression of the second cytotoxic peak^3. On the other hand, the short-term assays of CTL-mediated CMC show single-hit kinetics^5, perhaps comparable to part of the early CMC peak in our system. During our studies of immunity to syngeneic mammary tumours, we observed that when C3H or C3Hf immune CTL were tested against allogeneic MTV-induced mammary tumour targets, a relative degree of H-2 restriction of CMC was observed^2, although not of the magnitude detected in other systems using mostly short-term CMC assays$^{6-11}$. The experiments reported here show that when the kinetics of the CMC response against adherent syngeneic and allogeneic mammary tumour targets were compared, some differences became apparent: whereas the early CMC peak showed absolute H-2 restriction, no restriction was observed in the late CMC peak, although both events were mediated by Ly23 CTL (ref. 3).

Fig. 1 Cell-mediated cytotoxicity (CMC) of lymph node (LN) cells from C3Hf mice (C3H/Umc foster-nursed on C57Bl females, see ref. 1 for details) immunised with C3H/Umc (C3H) mammary tumour cells against syngeneic and allogeneic mammary tumour target cells, shows absolute H-2 restriction of the early CMC peak (2–8 h) and relative H-2 restriction of the late CMC peak (22–26 h). All tumours induced by the mouse mammary tumour virus (M-MTV) have appeared spontaneously and are kept *in vitro* using techniques described in ref. 1. Tumours tested were: C3H/Umc (MT.1 used for immunisation), C3H/HeJ (both have the *H-2 kk* haplotype similar to the LN cell donors), A/J (*H-2 kd*), RIII (*H-2 rr*) and (I × C57Bl/6)F$_1$ (*H-2 jj/bb*). The immune C3Hf LN cells were obtained from mice immunised by temporary growth in the footpad of a C3H/Umc mammary tumour (MT.1), followed by amputation 15–20 d later and collection of the regional LN 7–9 d after amputation^3. Immune LN cells were tested for CMC using ^{3}H-proline-pre-labelled adherent target cells (1,000 cells per well of microtest plates II, Falcon Plastics) and 100:1 effector:target ratios. The mixtures were incubated for the indicated times at 37 °C in humidified 5% CO$_2$ in air and the per cent cytotoxicity calculated as described in ref. 3 in relation to the target cells incubated with non-immune C3Hf LN cells (based on the measurement of the remaining adherent target cells per well after washing in experimental and control groups). For further details on cell preparation, target labelling and recovery of labelled adherent cells, see ref. 3. ●, C3H; ○, C3H/He; △, A; ▲, RIII; □, (I × C57BL/6)F$_1$.



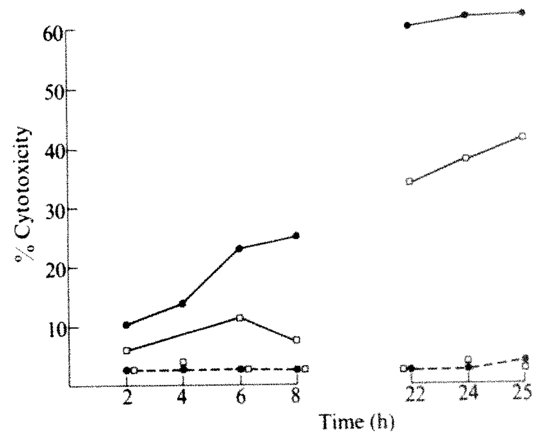


Fig. 2 Abrogation of early and late CMC of specifically immune C3Hf LN cells against syngeneic and allogeneic mammary tumour targets, by pretreatment of the effector LN cells with anti-Ly-2.1 and rabbit complement (C). Ly2 is an antigen expressed on T cells^{3,4}. For details on the CMC assay using ^{3}H-proline-labelled adherent target cells, *in vivo* immunisation and *in vitro* treatment of effector cells with anti-Ly sera, see ref. 3. Treatment with anti-Thy-1.2 and C also produced abrogation of both CMC peaks (data not shown, see also ref. 1). Conversely, removal of B cells by pretreatment with rabbit anti-mouse Ig and C had no effect on CMC; thus, at 24 h CMC was 59% in the syngeneic and 40% in the allogeneic target. Similarly, the addition of rabbit anti-mouse Ig (or control rabbit serum) at concentrations of 100 and 200 μg of Ig per culture had no effect on the early or late CMC peaks on the syngeneic or allogeneic target (data not shown). The addition of heat-aggregated goat IgG (at a concentration of 50 or 100 μg per ml of culture media) also had no effect on the early or late CMC peaks (data not shown). These additional studies confirm the view that both CMC peaks on syngeneic or allogeneic targets are mediated by T-effector cells. ●, C3H; □, (I × C57BL/6)F$_1$; —, control; - - -, anti-Ly-2.1 + C.

The early CMC peaks (2–8 h, Fig. 1) produced by C3Hf immune CTL against syngeneic C3H or H-2-identical (C3H/He) targets were comparable, whereas CMC was markedly reduced against the allogeneic targets. The late peak (22–26 h, Fig. 1) also seemed to show some degree of H-2 restriction^2. Figure 2 shows comparable data for the CMC response against C3H and (I × C57B1/6)F$_1$ targets during the early and late peaks, but also includes the abrogation of such responses by pretreatment of the CTL with Ly-2.1 serum and C, confirming our previous observation that both CMC peaks are mediated exclusively by Ly23 CTL (ref. 3). Figure 3 shows that when the whole time course of the response of C3Hf CTL against syngeneic or allogeneic mammary tumour targets was measured, it was evident that the late peak, appearing at 18 h, was quite comparable in magnitude and kinetics between the allogeneic and syngeneic targets. Thus, the relative H-2 restriction observed in the late peak in Fig. 1 was due to the absence of the early H-2-restricted CMC peak. It can be concluded that although the early CMC peak in the present model system shows absolute H-2 restriction, no such restriction is observed for the late CMC peak.

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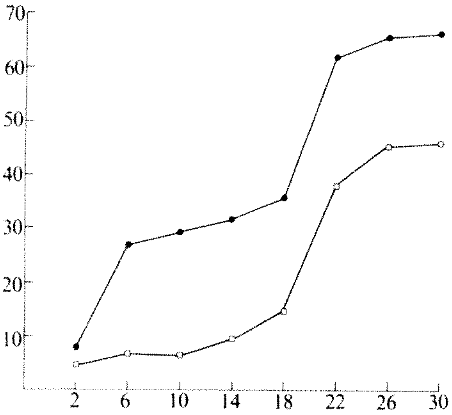

Fig. 3 Time course of CMC of C3Hf specifically immune LN cells against syngeneic (●) and allogeneic ((I × C57BL/6)F_1, ☐) mammary tumour target cells, showing absolute H–2 restriction of the early CMC peak (2–18 h) and no H–2 restriction of the late CMC peak (22–30 h), as the shape and magnitude of the late curve are comparable for the syngeneic and allogeneic targets. The difference in total CMC at 30 h between the allogeneic and syngeneic targets which appears as relative H–2 restriction is due to the absence of the early CMC peak in the allogeneic combination. For details on immunisation and assay system see Fig. 1 and ref. 3.

The interpretation of the present results requires some definitions concerning the assay and the system[3] in relation to the H–2 restriction model[6–8]. CTL generated *in vivo* in the local lymph node after hyperstimulation (temporary syngeneic tumour growth and removal) show specific *in vitro* CMC directed against syngeneic (or allogeneic) mammary tumour targets that requires relatively long-term assays for detection[1–3]. The kinetics of this assay indicate that complex events operate in the generation of the CMC response and that non-cytotoxic helper or amplifier T cells are required for the expression of maximal CMC[3]. Based on the timing of the response it is probable that the second peak of activity represents tertiary stimulation of memory cells into CTL, as observed in syngeneic and allogeneic systems[12,13], whereas the initial peak may represent CMC comparable to the short-term assays in other systems, and a possible measure of conventional CTL activity[5].

If this sketchy interpretation is accepted, several hypotheses may explain the observed non-restriction of the late peak. (1) Ly1 helper cells. As Ly1 helper–amplifier cells are required for the generation of the non-restricted second peak[3], it is possible that specific Ly1 help against the H–2–MTV complex in the allogeneic targets is generated during the long-term assay and that such help can by-pass the H–2-restricted activity of the Ly23 CTL. (2) Memory is non-restricted. Most of the literature on responses to viral and other antigens does not support this hypothesis[12–17]. In general, syngeneic cells bearing the appropriate antigen can induce good levels of CTL regeneration in secondary or tertiary stimulation[12–14], although H–2 identity may not be an absolute requirement[15–16]. However, it is accepted that the pattern of CMC of secondary cells is similar to that of the primary CTL[12–17]. (3) Changes in avidity of CTL. In allogeneic systems, it has been shown that avidity of CTL is influenced by time and antigen dose, in terms of antigen-driven selection of pre-cytotoxic T-cell clones with a spectrum of affinities for antigen[18]. Thus, cells with high-affinity receptors would be stimulated preferentially by antigen, giving rise early in the response to a low number of CTL with high affinity (and susceptible to H–2 restriction?). Later, cells with lower affinity would be stimulated, especially when the amount of antigen is not limiting, generating CTL with less avidity (and perhaps without H–2 restriction). Some of the kinetic data of the allogeneic responses fit well with the timing described in our assay for the generation of the second CMC peak[18], the putatively less avid, non-H–2-restricted tertiary CTL response. (4) Genetics of restriction. Cross-reactive lysis of hapten-modified targets has been observed when certain mouse strain combinations are tested[19]. One possible explanation of our data could be that secondary or tertiary activation of CTL in our system may show cross-reactive lysis (perhaps also under genetic control).

Several other explanations may account for the observed differences. These could include: (1) intrinsic properties of the assays (that is, most of the H–2 restriction has been shown with short-term assays, and in fact, no restriction was observed when longer assays were used[15,20–23]; (2) properties of the adherent mammary tumour lines or changes in H–2 expression during the assay (an inverse relationship between expression of MTV antigens and D-end specificities of H–2 has been observed[1]); (3) part of the CMC in our system is not mediated by T cells (this point has been eliminated by the experiments described in Fig. 2); (4) our system belongs to a non-restricted category[20–23]. Experiments to decide between some of these interpretations are in progress.

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Complete protection from low-dose streptozotocin-induced diabetes in mice

STREPTOZOTOCIN (SZ) is a broad-spectrum antibiotic with oncolytic, oncogenic and diabetogenic properties[1–4]. To produce diabetes in experimental animals, SZ is administered as a single bolus, injected either intravenously (i.v.) or intraperitoneally (i.p.). Within 72 h, selective destruction of pancreatic beta cells and hyperglycaemia occur[3,5]. Subdiabetogenic doses of SZ cause only mild degranulation of the β-cells without hyperglycaemia during the first 72 h, unlike multiple (five) subdiabetogenic (i.v. or i.p.) doses of SZ to Charles River Laboratory (CD-1) mice, which produce marked glucose elevations within 5–6 days of the last injection of SZ, associated with morphological evidence of insulitis[6,7]. To dissociate direct β-cell toxicity of SZ from the delayed appearance of the insulitis and hyperglycaemia, we used antilymphocyte serum and 3-*O*-methyl-D-glucose (3-OMG), a nonmetabolised glucose analogue, which prevents the occurrence of hyperglycaemia for at

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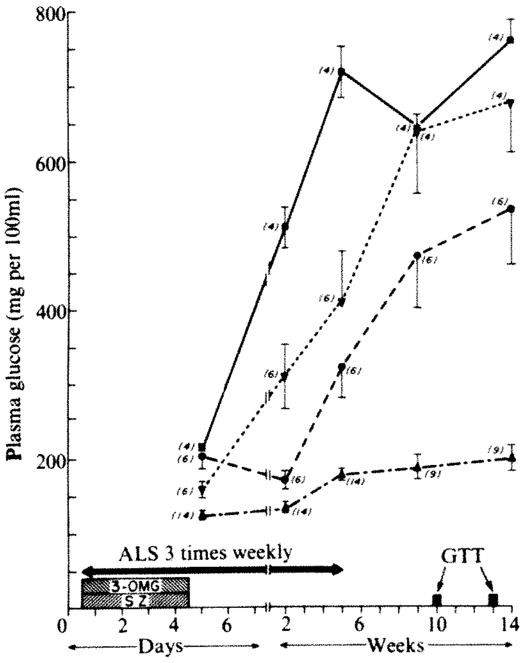
least 72 h, when administered to mice or rats prior to a single diabetogenic dose of SZ[8,9]. Antilymphocyte serum (ALS), while not altering the multiple subdiabetogenic SZ-induced hyperglycaemia, does protect against the lymphocytic infiltration of the pancreatic islets, without changing the delayed hyperglycaemic response observed following the combination of 3-OMG and SZ injections[10]. We report here that injections of ALS (for 5 weeks), in combination with a regimen of 3-OMG and SZ, prevented the development of hyperglycaemia for the entire 14 weeks of the experiment, and that mice demonstrated essentially normal intraperitoneal glucose tolerance tests and pancreatic histology during the 3rd and 4th months following SZ.

Four groups of mice were studied. Group 1 ($n = 4$) received five daily i.p. injections of SZ (40 mg per kg body weight). Group 2 ($n = 6$) received five daily injections of SZ and, in addition, i.p. ALS, 0.25 ml three times daily for a total of 5 weeks, including the period when SZ was administered. Group 3 ($n = 6$) received i.p. 3-OMG (1g per kg) before each of the five SZ injections. Group 4 ($n = 14$) received the combination of 3-OMG plus SZ and ALS.

Figure 1 represents the results of plasma glucose (PG) determinations. Mice receiving multiple injections of SZ alone (Group 1) showed an increase in plasma glucose (PG) by the second week. Mice injected with the combination of SZ and ALS (Group 2) had lower PG concentrations during the period of ALS administration, afterwards increasing to levels comparable to the group that received SZ alone. The group that received 3-OMG plus SZ (Group 3) showed no increase in PG for approximately 2 weeks, then PG rose to a level slightly lower than that observed in animals that received SZ alone or SZ plus ALS. In marked contrast to these three groups, the mice injected with the combination of SZ, 3-OMG and ALS (Group 4) remained normoglycaemic for the entire 14 weeks of the experiment.

Glucose tolerance tests for Group 4 were not significantly different from those of the normal controls (Fig. 2). Histological examination (9th week ($n = 5$) and the 14th week ($n = 9$)) of the pancreatic islets revealed subtle architectural disarray, occasional foci of infiltrating mononuclear cells, and mild β-cell degranulation at both time intervals. Islets from Group 4 did not show the marked lymphocytic infiltration or β-cell destruction observed previously in mice receiving SZ alone[6,7].

Fig. 1 Plasma glucose levels (mean ± s.e.m.) for 14 weeks in CD-1 mice after: five daily i.p. injections of SZ (40 mg per kg) (■); ALS injections (0.25 ml) three times per week for 5 weeks plus SZ (▼); 3-OMG (1 g per kg) before each SZ dose (●); 3-OMG, plus SZ plus ALS regimen (▲). Numbers in parentheses indicate the number of mice in each group. The mice, histological examination, collection, and assaying glucose levels, preparation and administration of 3-OMG, SZ, and ALS, are as previously described[10]. GTT, i.p. glucose tolerance test.



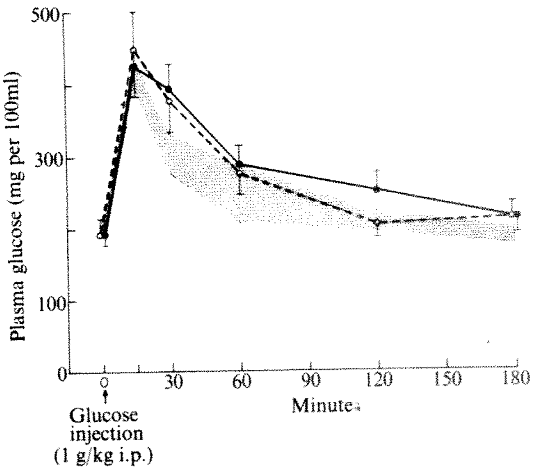


Fig. 2 I.p. glucose tolerance test. Plasma glucose response following i.p. injection of glucose (1 g per kg) in a volume of 1 ml, in animals which received the combination of 3-OMG, SZ and five weeks of ALS. The tests on experimental animals were performed during the 10th week and 13th week of the experiment. Also shown are the plasma glucose levels of five normal mice given i.p. glucose (1 g per kg) (stippled line). Numbers in parentheses indicate the number of mice in each group. ○, At 10 weeks ($n = 9$); ●, at 13 weeks ($n = 9$).

A plausible explanation of the protection provided by 3-OMG against the β-cell toxicity of SZ is that of direct competition between 3-OMG and SZ for a surface receptor site on the β cell[9]. In mice, a single injection of 3-OMG (2 g per kg) prior to SZ (up to a dose of 180 mg per kg) prevents β-cell necrosis and diabetes for up to 72 h[8]. The quantity of 3-OMG used in the present experiments should provide complete protection against each of the five injections of SZ (40 mg per kg). However, as previously observed[6], the administration of 3-OMG (1 g per kg) immediately before each of five consecutive daily doses of SZ (40 mg per kg) provided protection against glucose elevations for only approximately 2 weeks, after which the animals became diabetic, which correlated with the presence of insulitis[10]. We suggest that either 3-OMG is unable to protect the β cell completely from direct SZ toxicity, or that SZ initiates a response which is only partially affected by 3-OMG preinjection.

SZ-treated animals were given ALS to see if insulitis and/or hyperglycaemia could be attenuated by prolonged immunosuppression. In previous experiments, ALS administered for 10 consecutive days (that is, during the five daily injections of SZ, plus 5 days thereafter) produced no appreciable diminution in plasma glucose levels when compared with animals receiving SZ alone, However, the ALS-treated mice showed no insulitis and the combination of 3-OMG, SZ and ALS (10 injections) did not provide additional protective effect beyond that attributable to the five daily doses of 3-OMG alone[10]. The occurrence of diabetes in the ALS-treated animals might be considered paradoxical if our hypothesis of an immunological basis for the multidose SZ diabetes syndrome is correct unless the immunosuppression by ALS was inadequate to prevent the delayed β-cell destruction.

In the present study and in others using the inbred C57BL/KsJ mouse ($n = 10$) (data not shown), more prolonged administration of ALS (5 weeks) prevented diabetes. One explanation is that in the mice treated with ALS for only 10 days, the autoimmune state capable of destroying the β cells, was only temporarily suppressed by the ALS treatment. In contrast, the prolonged injection of ALS for five weeks suppressed the autoimmune state to a greater extent, islet (β cell) injury was minimised and physiological impairment of glucose tolerance did not occur during the 14 weeks of the experiment.

If direct β-cell toxicity by SZ is not the only mechanism by which the animals eventually became diabetic, the protection afforded by ALS at any time would be difficult to explain. We can assume that ALS must have protected the mice against the destructive effects of the postulated cell-mediated immune reactions against the β cell. The immunosuppressive mechanism of β-cell destruction remains to be elucidated.

SZ-induced diabetes can be ameliorated by simultaneous administration of 3-OMG and prolonged administration of ALS. We suggest that 3-OMG reduces the direct β cytotoxicity of SZ, and permits the antilymphocyte effects of ALS to be expressed by inhibiting the inflammatory islet lesions and the diabetic syndrome. Our results strengthen the hypothesis that cell-mediated autoimmunity plays a role in the pathogenesis in the model of SZ-induced insulitis.

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Sulphonylureas increase the number of insulin receptors

SULPHONYLUREA drugs have been widely used in the management of the form of diabetes mellitus which develops in adulthood. Although some endogenous insulin secretory capacity seems to be necessary for sulphonylureas to be effective (they are ineffective in pancreatectomised animals), their precise mechanism of action has not been clear. Short-term administration of sulphonylureas to animals or humans is associated with increased insulin secretion[1,2]. However, prolonged administration reduces pancreatic insulin content in animals[3,4] and diminished insulin secretion in reponse to nutrient stimulation in both laboratory animals[5] and humans with adult-onset (insulin-independent) diabetes[6,7]. Treatment with sulphonylureas also decreases the biosynthesis of proinsulin[8]. Thus, the chronic antidiabetic action of sulphonylureas does not seem to result from an effect on insulin release, but rather from one or more extrapancreatic effects[9,10]. In that connection, we have shown that chronic administration of the sulphonylurea, glipizide, to patients with adult-onset diabetes mellitus results in significant potentiation of insulin action, as assessed by changes in insulin-mediated glucose disposal[11]. On the basis of those results, we have suggested that glipizide influences the interaction of insulin with the cell membranes in insulin-sensitive tissues. We now report that, in an animal model, such an effect involves an increase in the number of insulin receptors.

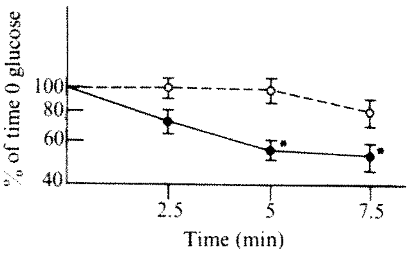

Fig. 1 Potentiation of insulin action by glipizide treatment in normal mice. 15–20-g male mice were treated with diet + glipizide (5 mg per kg per d) or diet alone for 10 d. Following a 5-h fast, the mice were injected intravenously with 2.5 mU pork insulin and killed at the specified times for measurement of plasma glucose. Each data point is the mean ± s.e. of five observations. The data are expressed as % of plasma glucose at time 0. Insulin had no effect on the plasma glucose in the control mice, but caused significant hypoglycaemia in the glipizide-treated mice at 5 and 7.5 min. ○, Control; ●, glipizide; * $P < 0.05$.

Male Dublin strain mice (15–20 g) were fed measured amounts of a standard diet. Glipizide (5 mg per kg per d) was added to the diet of half of them for 10 d. Weight and content of glucose and insulin in the plasma during fasting were not significantly different in controls and in mice receiving glipizide. However, sensitivity to insulin was enhanced by glipizide (Fig. 1); 2.5 mU of pork insulin did not significantly alter the plasma glucose of controls, but significant hypoglycaemia occurred in glipizide-treated mice 5 min after administration of insulin. Moreover, the concentration of insulin in the plasma at each of the times shown in Table 1 demonstrates that glipizide had no effect on insulin degradation and that insulin action was potentiated at physiological insulin concentrations. Thus, treatment of normal mice with glipizide potentiated the action of insulin on glucose disposal in a similar manner to that demonstrated in humans with diabetes mellitus.

We next investigated whether this potentiation of insulin action was accompanied by a change in the characteristics of insulin binding to the membranes of insulin-sensitive tissue. A second group of mice was given a standard diet with or without glipizide. After 10 d the livers were removed and homogenised, and purified plasma membranes were isolated by ultracentrifugation on a 15–55% linear sucrose gradient. Fractions

Table 1 Plasma insulin in mice following an intravenous dose of 2.5 mU of regular insulin

Time (min)	Glipizide treatment (μU ml^{-1})	Control (μU ml^{-1})
0	<2.5	<2.5
2.5	27.3 ± 4.2	30.5 ± 6.8
5	14.4 ± 2.3	11.0 ± 1.4
7.5	5.8 ± 1.0	7.0 ± 1.5

Values are mean ± s.e. of five animals.

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containing plasma membranes were identified by measurement of 5′ nucleotidase activity[12], and those containing endoplasmic reticulum were identified by measurement of NADH oxidase activity[13]. Protein concentrations were determined by the method of Lowry[14]. The specific activity of 5′ nucleotidase in the pooled plasma membrane fractions was 15 times that in the crude homogenate. Enzyme profiles and specific activities were identical for the livers of all mice. Insulin binding to plasma membranes was determined by incubation of membranes for 15 h at 4°C with biologically active ^{125}I-insulin and various concentrations of unlabelled insulin[15].

Figure 2 shows a representative experiment comparing (by Scatchard analysis[16]) insulin binding to purified hepatic plasma membranes from a glipizide-treated mouse with binding from a control mouse. The shapes of the two curves are similar, indicating that glipizide did not alter affinity for insulin. In contrast, the liver membranes of the treated mouse bound twice as much insulin as those of the control. ^{125}I-growth-hormone binding was assessed in the same way, using samples of liver membrane from the preparations already used for insulin binding. There was no difference between control and glipizide-treated mice in growth-hormone binding or displacement.

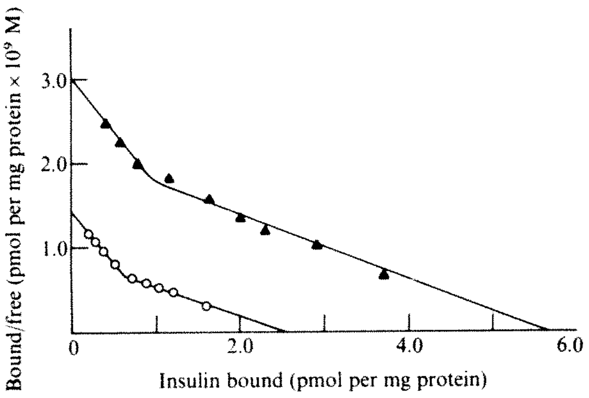

Fig. 2 Scatchard plot of insulin binding by purified hepatic plasma membranes from a glipizide-treated mouse and a control mouse. Binding studies were done by the method of Kahn *et al.*[15]. ○, Control; ▲, glipizide.

Table 2 summarises data calculated from four experiments similar to that depicted in Fig. 2. The Scatchard plots are curvilinear, including either negative cooperativity, binding sites of different affinities, or both. To facilitate analysis, we assumed that the first part of the curve represented a high-affinity binding site and the second part a low-affinity site. However, our conclusions do not depend on a choice between alternative mechanisms for curvilinearity. There is no significant difference in the affinity constant (K_a) between the control and glipizide-treated mice for either the high or low affinity receptors, but glipizide-treated animals have more than twice as many receptors.

Our findings provide a basis for understanding the effects of glipizide on patients with adult-onset diabetes mellitus. These patients have a marked resistance to insulin action[17], which is probably a primary defect. Although the mechanism involved has not been elucidated, abnormally low numbers of insulin receptors have been found in such insulin-resistant states as adult-onset diabetes[18] and obesity[19]. Olefsky and Reaven reported that when plasma glucose was decreased by treatment with sulphonylurea in a group of adult-onset diabetic patients, the number of insulin receptors on their circulating monocytes increased[18]. Our observation that successful treatment with glipizide is associated with a potentiation of insulin action on glucose disposal[11] is consistent with a glipizide-induced increase in the number of available insulin receptors on the plasma membranes of responsive tissues. Our data suggest that this effect is not secondary to a decrease in plasma glucose.

Table 2 Effect of glipizide treatment on purified hepatic plasma membrane insulin receptors

	High affinity receptor		Low affinity receptor	
Treatment	Receptor no. (pmol per mg)	K_a (10^9 m^{-1})	Receptor no. (pmol per mg)	K_a (10^9 m^{-1})
None	1.4 ± 0.1	1.0 ± 0.2	2.1 ± 0.1	0.4 ± 0.1
Glipizide (5 mg per kg per d)	3.2 ± 0.6*	0.9 ± 0.2	4.6 ± 1.0*	0.4 ± 0.1

Data are mean ± s.e. of four separate experiments.
* $P < 0.05$.

Our results suggest that *in vivo* glipizide treatment increases the number of insulin receptors on the target cell membrane, enhancing sensitivity to insulin[20]. The number of plasma membrane insulin receptors *in vivo* is influenced by many factors, and our data do not enable us to delineate the precise way in which glipizide acts. Possibilities under study include direct effects on the plasma membrane, an increase in target cell cyclic AMP, which might increase the number of insulin receptors[21], or an alteration in the mechanism by which insulin modifies the characteristics of its own receptor.

Although our results represent an important step towards understanding the mechanism of action of sulphonylurea drugs, their clinical value can be determined only by analysis of epidemiological studies comparing long-term benefits and risks. We do not know whether sulphonylureas increase insulin receptors and insulin sensitivity in all patients with adult-onset diabetes or in only a subset of patients, and the duration of the effect is unknown. Our data do, however, have practical importance in guiding the development of new drugs which influence membrane function and insulin action and in amplifying the concept of insulin resistance as a major metabolic defect in adult-onset diabetes mellitus.

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Tolerance of locus coeruleus neurones to morphine and suppression of withdrawal response by clonidine

NORADRENERGIC neurones of the locus coeruleus (LC; A6 of ref. 1) are inhibited by the systemic or local administration of adrenergic agonists[2-5], opiates[6,7] and enkephalins[8,9]. The adrenergic receptors of the LC, which are of the presynaptic or α_2 type[5], seem to mediate inhibitory responses to recurrent LC collaterals[10] and adrenaline inputs from the lower brain stem[4,11]. In addition to adrenergic receptors, there are opiate receptors located in the LC[12,13] which presumably mediate the actions of endogenous opiate-like substances (enkephalins and β-endorphin) located in nerve terminals within the LC[14-16]. Recently, clonidine, which is the most powerful of the α_2 agonists known to inhibit the firing of LC neurones[3,5], has been reported to suppress the symptoms of opiate withdrawal in humans[17]. On this basis, the latter authors suggested that the opiate-withdrawal syndrome may be due in part to increased noradrenergic neuronal activity in areas such as the LC which are regulated by both α_2 adrenoceptors and opiate receptors. In this connection, it is interesting that the drug piperoxane, which blocks α_2 adrenoceptors and accelerates the firing of LC neurones[4,5] produces many of the symptoms (such as anxiety and hypertension) seen in opiate withdrawal[18]. In the present study, single-cell recording and microiontophoretic techniques were used to establish the development of tolerance of LC cells to the depressant effects of morphine, naloxone-induced withdrawal activations of LC neuronal firing in morphine-dependent animals, and the ability of clonidine to suppress the withdrawal of LC neurones through a non-opiate receptor.

Thirty male albino rats (Charles River) weighing 225–275 g were used. The animals were anaesthetised with chloral hydrate (400 mg per kg, intraperitoneally) and mounted in a stereotaxic instrument. Morphine tolerance and dependence were induced by the method of subcutaneous pellet implantation[19,20]. The pellets, which contained 75 mg of morphine base, were implanted daily for 1–5 d. For the single LC-cell recordings, a burr hole was made 1.2 mm posterior to lambda and 1.1 mm lateral to the midline. As described previously[2-6,10], practical aids in finding the LC include: (1) depth 5.5–6.5 mm below dural surface; (2) a zone of relative electrical silence ventral to the cerebellum and dorsal to the LC corresponding to the fourth ventricle; (3) the presence just lateral to the LC of cells of the mesencephalic nucleus of the fifth nerve, which are activated by displacement of the mandible; (4) in the LC itself, a closely packed population of slowly firing cells all responding to noxious stimulation by a burst of firing followed by a long quiescent period (~1 s). Ultimately, the recording sites were marked at the end of each experiment by iontophoretically ejected dye (Pontamine sky blue) for histological confirmation of LC and other recording sites.

Procedures for single-cell recording and microiontophoresis were as described previously[5]. The 5-barrel micropipettes which were used were broken to a tip size of 3–5 μm; impedances of the recording (central) barrels were 3.5–5.5 MΩ (filled with 2 mM NaCl containing 2% Pontamine sky blue). Solutions for microiontophoresis were as follows: morphine sulphate, 0.1 M, *p*H 4 (Merck); piperoxane, 0.1 M *p*H 4 (Rhone-Poulenc); clonidine HCl, 0.01 M in 0.1 M NaCl, *p*H 4 (Boehringer-Ingelheim); naloxone HCl, 0.05 M, *p*H 4 (Endo). Note that, because of its extreme potency in inhibiting LC neurones, the clonidine was ionically diluted with NaCl to reduce its transport number[3]. A retaining current of −10 nA was applied to all drug barrels between periods of ejection. Per cent inhibition was determined by the average rate for the 1-min period immediately following iontophoretic ejection; baseline rate was taken from the 1-min period just before ejection.

The firing of LC neurones slowed gradually and became totally suppressed within 1–2 h of implantation of a single morphine pellet (*n* = 4 animals); activity remained suppressed for about 24 h, and returned towards normal rates by 48–72 h. This pattern is consistent with the fact that morphine is released most rapidly from implanted pellets within the first 24 h[19,20]. LC cells could be activated by a noxious stimulus during periods of morphine inhibition, as has been reported previously[6]. Twenty-four hours after the last of 4–5 daily pellet implantations, the firing of randomly encountered LC neurones (mean rate 1.2 spikes s^{-1}, *n* = 20 cells) was nearly as high as in control animals (mean rate 1.4 spikes s^{-1}, *n* = 20 cells). The difference between LC firing rates in chronic morphine-treated and control animals was not statistically significant ($P > 0.1$, Student's *t* test), indicating that the morphine pellet implantation procedure had induced a considerable degree of tolerance. In control animals, the microiontophoretic application of morphine at a low ejection current (+5 nA) produced marked inhibitory effects (87% mean depression, *n* = 10 cells) on LC neurones; at low ejection currents naloxone totally blocked these effects of morphine but did not increase baseline firing rates (Fig. 1*a*).

At a low ejection current (+5 nA) clonidine caused a similar degree of depression in LC cell firing (91% mean depression, *n* = 10 cells) despite the fact that the effect of morphine had been totally blocked by the concurrent microiontophoresis of naloxone (Fig. 1*a*). Conversely, the α antagonist piperoxane blocked the effect of clonidine without altering the response to morphine

Fig. 1 The effects of microiontophoretically applied morphine and clonidine on the firing of LC neurones in control and morphine-dependent rats. In *a*, taken from a recording in a control animal, the initial marked inhibitory effect of morphine (MOR) was totally blocked by naloxone; clonidine (CLON) had its usual inhibitory effect even in the presence of naloxone. In *b*, also taken from a control animal, piperoxane (PIP) selectively blocked the inhibition produced by clonidine; the response to morphine after piperoxane was virtually identical to the original response. In *c*, taken from a morphine-dependent animal (24 h after five consecutive daily pellet implantations), the initial inhibition produced by morphine was only about 50%; an activation of firing was induced by naloxone iontophoresis, during which time the inhibitory effect of morphine but not clonidine was blocked. The records represent average rate histograms generated by the analog output of an electronic counter (10 s reset time). Periods of microiontophoretic ejection are indicated by bars above the rate records; the numbers above bars give ejection currents in nA.

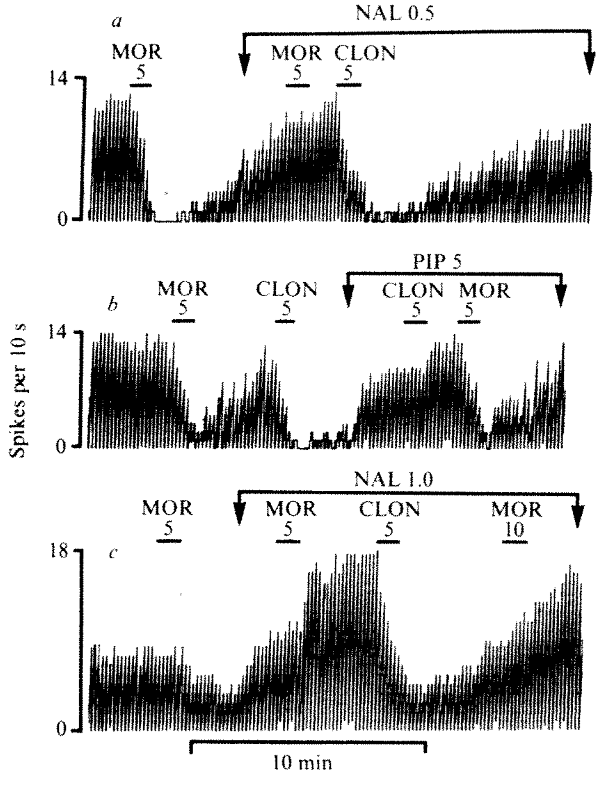

Table 1 Incidence of neuronal responses to microiontophoretically applied morphine and naloxone in LC and adjacent areas of control and morphine-dependent rats

Preparation	Region (no. of cells)	Morphine		Naloxone	
		% Cells activated	% Cells depressed	% Cells activated	% Cells depressed
Control rats ($n = 5$)	Locus coeruleus ($n = 10$)	0	100	0	0
	Cerebellum, Purkinje cells ($n = 6$)	33	0	0	0
	Mesencephalic nucleus of fifth nerve ($n = 5$)	0	0	0	0
	Reticular formation/central grey ($n = 10$)	10	10	0	0
Morphine-dependent rats ($n = 5$)	Locus coeruleus ($n = 10$)	0	100	100	0
	Cerebellum, Purkinje cells ($n = 5$)	40	0	0	0
	Mesencephalic nucleus of fifth nerve ($n = 5$)	0	0	0	0
	Reticular formation/central grey ($n = 12$)	8	8	8	8

In all cases, microiontophoretic currents were: +5nA, morphine; +0.5–1.0 nA, naloxone. Morphine was applied for 1 min; naloxone was applied 3 or more min. Purkinje cells were identified by climbing fibre bursts. The mesencephalic nucleus of the fifth nerve was identified by activation caused by displacement of mandible.

($n = 5$ cells; Fig. 1*b*). Figure 1*c* shows that in morphine-dependent rats (24 h after 4–5 daily pellet implantations) the inhibitory response of LC cells ($n = 10$) to microiontophoretic morphine was reduced compared with the controls (54% compared with 87%; $P < 0.01$, Student's *t* test). In all the dependent animals, the sustained microiontophoresis of naloxone on to LC cells produced a dramatic increase over baseline firing rate (mean 126%, $n = 10$ cells; Fig. 1*c*). Following naloxone, responses to microiontophoretically applied morphine were abolished. However, even during periods of naloxone-induced opiate-receptor blockade and withdrawal activation, the microiontophoresis of clonidine was still able to depress LC cell firing to below baseline rates (Fig. 1*c*). The uniform responses of LC cells to morphine and naloxone in both control and dependent animals were in sharp contrast to the relatively low incidence of effects in cells in areas immediately surrounding the LC (Table 1).

The first part of this study shows that tolerance develops to the powerful inhibitory effect of morphine on the firing of LC neurones. In fact, 24 h after 4–5 daily morphine pellet implantations, LC cells fired in a normal range, whereas 24 h after a single pellet spontaneous activity was markedly suppressed. In tolerant animals the direct application of naloxone to LC neurones by microiontophoresis induced a withdrawal response characterised by a >100% activation of firing. In other experiments (not reported above) the intravenous administration of naloxone (0.02 mg per kg) to dependent (but not control) rats also produced a dramatic increase in LC cell firing; this increase was reversed by intravenous clonidine. However, because of the generalised nature of the withdrawal reaction produced by systemically administered naloxone, it cannot be known with certainty that the effects of LC cell firing represent a direct action within the LC. It has been reported that the iontophoretic application of naloxone to cortical neurones in morphine-dependent rats induces an increase in neuronal firing[21]. In the present study, at the same low microiontophoretic ejection currents which were used in the LC, most neurones in areas immediately surrounding the LC (cerebellum, mesencephalic nucleus of the fifth nerve, central grey/reticular formation) were not responsive to morphine and did not display naloxone-induced withdrawal. The marked sensitivity and high selectivity of LC neurones for opiates and opiate antagonists in this region of the brain is consistent with previous studies[6–8].

The results of this study also indicate that morphine and clonidine act at independent LC-cell receptors, as the opiate antagonist naloxone blocks morphine but not clonidine whereas the α antagonist piperoxane blocks clonidine but not morphine. Previous studies, in which morphine and D-amphetamine were administered by the systemic route, also suggest that opiate receptors and adrenergic receptors which regulate LC cells are distinct, as naloxone blocked the LC-inhibitory action of morphine but not that of the indirectly acting adrenergic agent D-amphetamine[6]. The present study extends this work by showing that specifically within the LC itself naloxone antagonises morphine but not the α_2-adrenergic agonist clonidine. In addition, reciprocal selectivity has been shown by the fact that the α blocker piperoxane antagonised clonidine but not morphine. Although morphine and clonidine thus seem to act on independent receptors within the LC, it is notable that they have similar depressant effects on overall LC cell activity. These observations provide a basis for the proposal that the α_2 agonist clonidine might suppress certain symptoms of opiate withdrawal by means of a parallel but independent action on cell activity[17]. Moreover, a perpetuation of a state of dependence is avoided as clonidine acts on a non-opiate receptor. Of course there are opiate receptors at diverse sites within the central nervous system[12,13,22], some of which presumably do not overlap with the location of α_2 adrenoceptors. Nevertheless, the effectiveness of clonidine in suppressing opiate withdrawal in humans suggests that significant components of the opiate-withdrawal syndrome may be due to increased noradrenergic activity in areas such as the LC which are affected in a like manner by both α_2 adrenoceptors and opiate receptors[17]. It is also possible that at some sites clonidine could suppress opiate withdrawal through an action on non-adrenergic receptors (for example, histamine receptors[23]). However, the actions of clonidine in the LC would seem to be through an α adrenoceptor as they can be blocked selectively by the α antagonist piperoxane.

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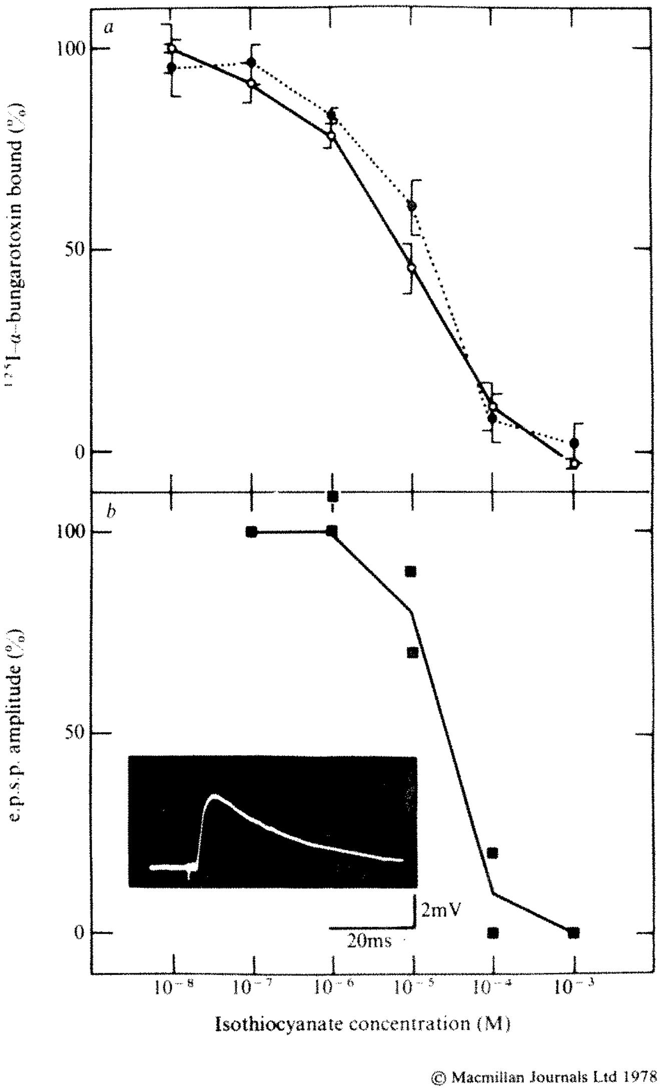
Insect acetylcholine receptors as a site of insecticide action

ACETYLCHOLINE is thought to be an excitatory neurotransmitter at synapses in the insect central nervous system (CNS) and does not seem to be involved in insect neuromuscular transmission[1,2]. Most of the current generation of insecticides are inhibitors of the enzyme acetylcholinesterase which hydrolyses acetylcholine, terminating its synaptic actions[3]. Far less attention has been given to other components of cholinergic synapses which might constitute potential sites of action of insecticidally active molecules. These include the presynaptic synthesis of acetylcholine involving the enzyme choline acetyltransferase, the mechanism of transmitter release and the postsynaptically located acetylcholine receptor molecules. The present study demonstrates that an isothiocyanate compound and nicotine, both of which show insecticidal activity, are agonists for an acetylcholine receptor in the CNS of the cockroach *Periplaneta americana*.

2-Isothiocyanatoethyltrimethylammonium iodide, synthesised as a potential insecticide, was found to be a potent inhibitor of choline acetyltransferase partially purified from fly heads[4]. In the same study the dissociation constant for the enzyme–inhibitor complex was found to be 0.06 μM. No significant inhibition of fly-head acetylcholinesterase was detected at a concentration of 100 μM (ref. 4). The possibility of a cholinergic presynaptic site of action in the insect CNS was therefore tested by perfusing the terminal abdominal ganglion of *Periplaneta* with saline containing 2-isothiocyanatoethyltrimethylammonium iodide (normal saline composition in mM: NaCl, 208.6; KCl, 3.1; $CaCl_2$, 5.4; $NaHCO_3$, 2.0; *p*H 7.0). Transmission at the cercal-nerve giant-fibre synapses, for which acetylcholine is the putative transmitter[5,6], was monitored by oil-gap (single-fibre) and sucrose-gap recording techniques[7]. A slow progressive block of the evoked excitatory postsynaptic potential (e.p.s.p.) might have been expected because of the time required both for access of the compound to the intracellular enzyme choline acetyltransferase and for the utilisation of existing stores of acetylcholine. Instead, however, a rapid reduction of the evoked e.p.s.p. and postsynaptic membrane resistance was detected, accompanied by depolarisation of the postsynaptic membrane at concentrations above 10^{-5} M (ref. 7). These results suggested a direct action of the isothiocyanate on the postsynaptic membrane, possibly on the acetylcholine receptors. The present study was designed to test this hypothesis by examining the binding of the isothiocyanate to an insect acetylcholine receptor and comparing this quantitatively with its ability to produce postsynaptic blockade. For comparison, parallel experiments were carried out using nicotine, which is both an insecticide and a potent ganglionic blocking agent in insects[5].

As a receptor probe we have used ^{125}I-α-bungarotoxin, which binds specifically to nicotinic acetylcholine receptors of vertebrates[8,9]. An ^{125}I-α-bungarotoxin-binding component with the expected properties of an acetylcholine receptor[10] has been characterised in fly heads of *Drosophila melanogaster*[11–14] and *Musca domestica*[13], in brain tissue of the moth *Manduca sexta*[15], and in abdominal nerve cords of *P. americana*[16]. In view of the controversy over the relationship between α-bungarotoxin-binding activity and the nicotinic acetylcholine receptor in the vertebrate CNS[17,18], it is important to note that α-bungarotoxin blocks transmission at the cercal-nerve giant-fibre synapses in the terminal abdominal ganglion of *Periplaneta*[19]. To determine whether the isothiocyanate and nicotine bind to acetylcholine receptors, we have compared their ability to

Fig. 1 *a*, Concentration dependence of inhibition by 2-isothiocyanatoethyltrimethylammonium iodide of ^{125}I-α-bungarotoxin binding to extracts of both whole flies (*D. melanogaster*) and cockroach abdominal nerve cords (*P. americana*). Results are expressed as a percentage of toxin bound to extract in the absence of the isothiocyanate. Each point is the average of three replicates and vertical bars denote 1 s.d. Concentrations for 50% inhibition of toxin binding are estimated to be 6.9×10^{-6} M (*Drosophila*; ○) and 1.6×10^{-5} M (*Periplaneta*; ●). *b*, Effects of various isothiocyanate concentrations on the amplitude of the evoked e.p.s.p. recorded from the cercal-nerve giant-fibre synapses in the terminal abdominal ganglion of *P. americana* using a modified sucrose-gap technique[7]. Concentration for 50% suppression of the e.p.s.p. is estimated to be 2.6×10^{-5} M. Inset shows the e.p.s.p. recorded in normal saline (100%).



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inhibit ^{125}I-α-bungarotoxin binding to particulate extracts with their competence to modify transmission at a synapse that can be blocked by α-bungarotoxin. Differential centrifugation techniques described previously have been used to prepare the particulate form of the receptor from *D. melanogaster*[11] (20,000*g* pellet from whole flies) and *P. americana*[16] (40,000*g* pellet from abdominal nerve cords). Extract equivalent to either 0.005*g* flies (*Drosophila*) or to 0.001*g* abdominal nerve cords (*Periplaneta*) was incubated with a solution of either 2-isothiocyanatoethyltrimethylammonium iodide or nicotine in Krebs original Ringer buffer, *p*H 7.4 (ref. 20) at 21° C for 30 min. Such mixtures were then combined with ^{125}I-α-bungarotoxin in Krebs Ringer buffer, *p*H 7.4, containing 0.1% bovine serum albumin and incubated in microfuge tubes (Beckman, no. 314326) at 21° C for a further 30 min, before centrifugation at 30,000*g* (30 min, 4° C).

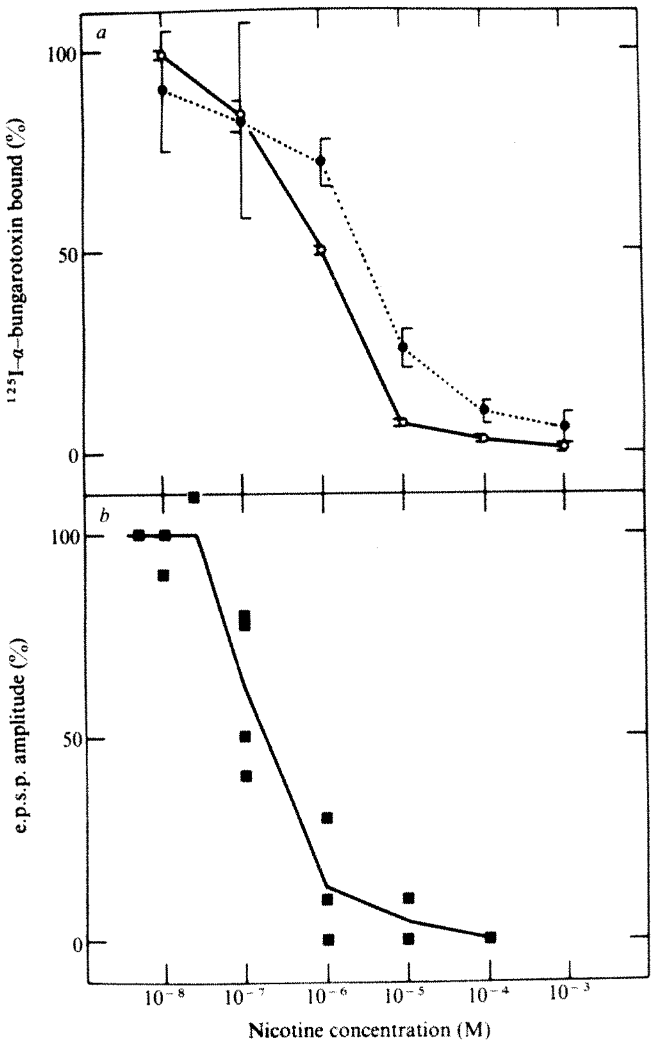

Fig. 2 *a*, Concentration dependence of inhibition by nicotine of ^{125}I-α-bungarotoxin binding to extracts of both whole flies (*D. melanogaster*) and cockroach abdominal nerve cords (*P. americana*). Results are expressed as percentage of toxin bound to extract in the absence of nicotine. Concentrations for 50% inhibition of toxin binding are estimated to be 1.0×10^{-6} M (*Drosophila*; ○) and 3.0×10^{-6} M (*Periplaneta*; ●). Each point is the average of three replicates and vertical bars denote 1 s.d. *b*, Effects of various concentrations of nicotine on the amplitude of the evoked e.p.s.p. recorded from the cercal-nerve giant-fibre synapses in the terminal abdominal ganglion of *P. americana*. Concentration for 50% suppression of the e.p.s.p. is estimated to be 2.0×10^{-7} M.

After removal of the supernatant fluid, pellets were rinsed twice and counted at 80% efficiency in a gamma counter (Searle model 1197). Background binding determined as the amount of ^{125}I-α-bungarotoxin bound to heat-inactivated extract was routinely subtracted. Similar levels of background binding were noted in the presence of 10^{-3} M nicotine. 100% binding was defined as the amount of ^{125}I-α-bungarotoxin bound in the absence of added ligand. In this way it was shown that 2-isothiocyanatoethyltrimethylammonium iodide inhibited toxin binding to the insect extracts (Fig. 1*a*). The concentrations of isothiocyanate producing 50% inhibition of toxin binding (ID_{50}) were estimated to be 1.6×10^{-5} M (*Periplaneta*) and 6.9×10^{-6} M (*Drosophila*). It was also demonstrated that nicotine inhibited toxin binding somewhat more effectively than the isothiocyanate (Fig. 2*a*). The ID_{50} concentrations for nicotine were estimated to be 3.0×10^{-6} M (*Periplaneta*) and 1.0×10^{-6} M (*Drosophila*). This value obtained for *Drosophila* extract agrees with the ID_{50} value of 0.5×10^{-6} M previously estimated for the inhibition by nicotine of toxin binding to *Drosophila* extract[11]. The ability of these insecticidally active compounds to inhibit α-bungarotoxin binding is similar to that found for acetylcholine in the presence of an anticholinesterase (ID_{50} = 2.8–7.9×10^{-6} M for *Drosophila*)[11].

In parallel electrophysiological experiments on the terminal abdominal ganglion of *Periplaneta*, changes were followed in the amplitude of the monosynaptic e.p.s.p. evoked by electrical stimulation of cercal nerves and recorded by the sucrose-gap technique from the cercal-nerve giant-fibre synapses during the perfusion of the ganglion by the test compounds. The ganglionic nerve sheath was routinely split to facilitate access to the synapses of the applied compounds[7]. It was estimated that 50% inhibition of the e.p.s.p. was produced by a concentration of 2.6×10^{-5} M isothiocyanate (Fig. 1*b*). As shown in Fig. 2*b*, the concentration of nicotine estimated to produce 50% block of the e.p.s.p. was 2.0×10^{-7} M. Just as nicotine was more effective than the isothiocyanate in inhibiting toxin binding *in vitro*, it was also a more potent inhibitor of the e.p.s.p

We have demonstrated that 2-isothiocyanatoethyltrimethylammonium iodide produces half-maximal block of ^{125}I-α-bungarotoxin binding to the insect extracts (*Periplaneta* CNS and *Drosophila* whole flies) at concentrations similar to those producing half-maximal suppression of e.p.s.p. amplitude at a central synapse in *Periplaneta* which can be blocked by α-bungarotoxin[19]. Differences between the factors affecting biochemical and physiological techniques make direct comparison of absolute ID_{50} values difficult. However, within each technique the order of effectiveness of test compounds can be determined and then compared between techniques. Studies with nicotine have shown that this cholinergic ligand is more potent than the isothiocyanate both in its ability to inhibit ^{125}I-α-bungarotoxin binding and in its actions on the postsynaptic membrane. The data indicate that the synaptic actions of both these insecticidally active molecules can be accounted for by their binding to the α-bungarotoxin-sensitive acetylcholine receptors, as they both inhibit toxin binding at physiologically relevant concentrations.

Our results provide direct evidence for an insect acetylcholine receptor as a site of insecticide action and thereby emphasise the need for detailed comparative studies on the properties of insect and vertebrate cholinergic receptors. We have demonstrated that the α-bungarotoxin binding assay could provide a rapid way to screen potential insecticides for action on a cholinergic receptor. We have also, indirectly, illustrated the difficulties in attempting a rational design of insecticide molecules. It should be recalled that 2-isothiocyanatoethyltrimethylammonium iodide, which has been shown to be effective at the postsynaptically located α-bungarotoxin-sensitive acetylcholine receptor, was originally designed as an inhibitor of the presynaptically located enzyme choline acetyltransferase.

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Morphine and β-endorphin influence the secretion of the endocrine pancreas

OPIOID PEPTIDES[1-3] are present in high concentrations in those areas of the brain and gastrointestinal tract[4] in which opiate receptors[5] are numerous and a biological response to opiates well established[6]. The report of immunoreactive enkephalin in the pancreas[4] raised the possibility that a similar association between the presence of endogenous peptide and opiate receptors might exist in this organ and would be manifested by a biological response to these agents. We report here that an exogenous opiate and an endogenous opioid peptide both exert biological effects upon the endocrine pancreas that are compatible with the presence of opiate receptors in the islets of Langerhans.

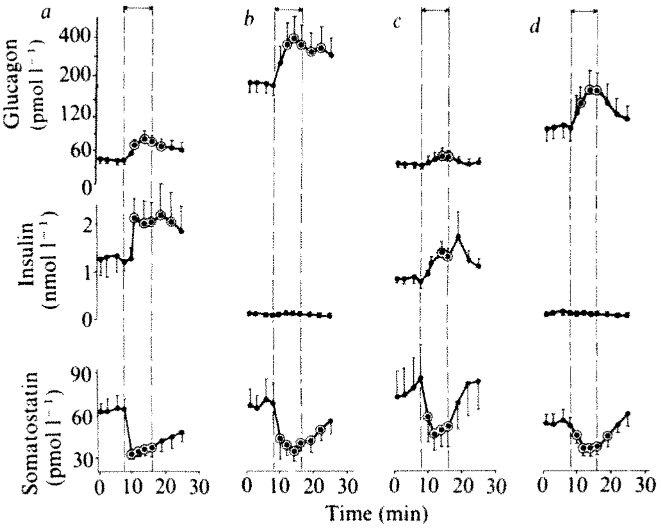
We examined the effects of morphine and the opioid peptide β-endorphin on the release of immunoreactive insulin, glucagon and somatostatin from the isolated perfused dog pancreas. We used the preparation of Iversen and Miles[7], in which the pancreas is perfused without recirculation at a rate of 20 ml min^{-1}. Somatostatin-like immunoreactivity (SLI) was measured by a radioimmunoassay using antiserum R101 of Arimura[8,9], and insulin and glucagon by a modification of previously described methods[10,11].

Perfusion of morphine sulphate (Wyeth) at a concentration of 3 μmol l^{-1} in the presence of 5.5 mM glucose lowered SLI levels by 50% ($P < 0.05$) at 2 min, while insulin and glucagon levels almost doubled ($P < 0.05$), each reaching a peak at 4–6 min (Fig. 1*a*). When glucose was omitted from the perfusate (Fig. 1*b*), SLI levels again declined by approximately 50% ($P < 0.05$); however, as expected, baseline glucagon levels were higher and the peak glucagon level was fourfold greater than in the presence of 5.5 mM glucose, while baseline insulin concentrations were lower and did not rise measurably in response to the morphine.

We then tested the effects on islet hormone secretion of the opioid peptide, β-endorphin (synthesised at the Salk Institute by N. Ling), at a concentration of 54 nmol l^{-1}, 1/50 of the molar concentration of morphine, both in the presence of 5.5 mM glucose and in its absence. As in the case of morphine, in the presence of 5.5 mM glucose SLI release was suppressed by 50% and again statistically significant inhibition occurred within 2 min ($P < 0.05$), preceding the peak changes in insulin and glucagon (Fig. 1*c*). Mean glucagon concentrations rose slightly but significantly ($P < 0.05$), and insulin levels increased almost twofold, with peaks once again occurring at 4–6 min ($P < 0.05$). Omission of glucose from the perfusate was again associated with higher baseline levels of glucagon and an exaggerated glucagon response to β-endorphin ($P < 0.05$), while baseline insulin levels were lowered and the insulin response to the opioid peptide abolished. However, SLI levels were suppressed ($P < 0.05$) as in the presence of glucose (Fig. 1*d*).

The similarity of the islet responses to two dissimilar substances, an opiate alkaloid and an endogenous peptide, suggested that they were mediated by a specific receptor mechanism. If so, these effects should be prevented by a specific antagonist. We therefore examined the effects of the opiate antagonist, naloxone hydrochloride (Endo), perfused at 11 μmol l^{-1}. Naloxone perfused alone did not affect either glucagon or SLI release, although insulin concentration increased significantly ($P < 0.05$) (ref. 9, Fig. 2*a*). Naloxone blocked the morphine-induced changes in glucagon and SLI, but the insulin response was reduced by only about 50% (Fig. 2*b*, left panel). The effects of β-endorphin on glucagon, somatostatin and insulin were completely abolished by the opiate antagonist ($P < 0.05$) (Fig. 2*b*, right panel).

Fig. 1 The effect of morphine (*a*, *b*) and β-endorphin (*c*, *d*) on immunoreactive glucagon, insulin and somatostatin release from the isolated dog pancreas. Note the change in scale on the glucagon axis. Significant differences ($P < 0.05$, ref. 9) from baseline are indicated by encircled dots. The pancreas was surgically isolated from healthy conditioned male dogs weighing 20–30 kg and then perfused with a semisynthetic buffer, as previously described[5]. The perfusate consisted of a Krebs–Ringer-bicarbonate buffer with 4% Dextran T-70 (Pharmacia), 10 mM arginine, with or without 5.5 mM glucose. Morphine sulphate or porcine β-endorphin were infused for 8-min periods on one or two occasions in each pancreas. The pancreatic effluent was collected at 1-min intervals and frozen for subsequent radioimmunoassay. *a*, 10 mM arginine + 5.6 mM glucose; morphine 3 μmol l^{-1}; $n = 6$. *b*, 10 mM arginine, no glucose; morphine 3 μmol l^{-1}; $n = 6$. *c*, 10 mM arginine + 5.5 mM glucose; β-endorphin 54 nmol l^{-1}; $n = 7$. *d*, 10 mM arginine, no glucose; β-endorphin 54 nmol l^{-1}; $n = 7$.



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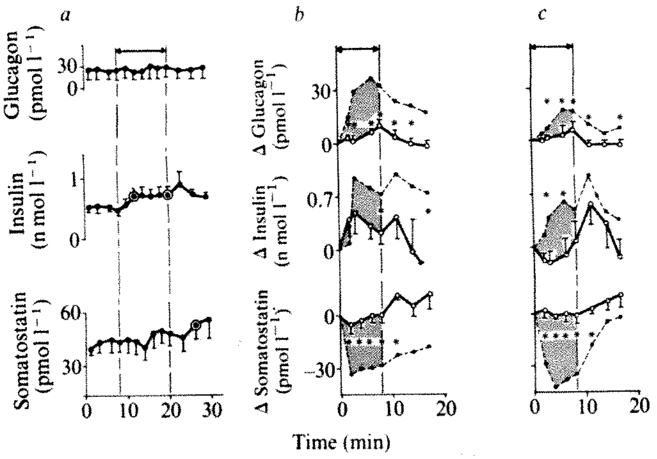

Fig. 2 *a*, The effect of naloxone alone on immunoreactive glucagon, insulin and somatostatin release from the isolated dog pancreas. Significant differences ($P < 0.05$, ref. 12) from baseline are indicated by encircled dots. *b*, The effect of naloxone on morphine and β-endorphin induced changes in islet cell function. Morphine (3 μmol l^{-1}) or β-endorphin (54 nmol l^{-1}) were perfused for 8 min, beginning 4 min after the start of the naloxone perfusion. The responses during naloxone perfusion are compared to those in the absence of naloxone, and the differences are indicated by the stippled areas. The hormone concentration immediately before the perfusion of morphine or β-endorphin was used as the baseline in order to derive the changes induced by each substance over and above changes induced by naloxone alone. Statistically significant ($P < 0.05$, ref. 13) differences between the two groups are indicated by an asterisk. The perfusate of all experiments illustrated in Fig. 2 contained 5.5 mM glucose, and each experiment was carried out in a different pancreas. *a*, 10 mM arginine + 5.5 mM glucose; naloxone 11 μmol l^{-1}. *b*, Morphine; ○, naloxone added; ●, no naloxone. *c*, β-Endorphin; ○, naloxone added; ●, no naloxone. Values are means from six experiments.

Thus, it seems that the opiate alkaloids and endogenous opioid peptides are capable of altering islet cell function, probably via a specific receptor mechanism. A similar observation has recently been made with respect to the exocrine pancreas[14], long known to be responsive to morphine[15]. The presence of endocrine cells with enkephalin-like immunofluorescence in islets of the pancreas[16] raises the possibility that locally secreted enkephalin may influence islet cell function through nearby receptors, and that circulating β-endorphin, which is released from the corticotrophs of the pituitary in response to stress[15], might also exert such effects. The failure of naloxone to produce any effect on basal SLI and glucagon release argues against a persisting 'tonic' *in vitro* action of endogenous opioid peptides that can be blocked by the antagonist, but does not exclude such activity *in vivo* in an intact pancreas.

Hyperglycaemia has been observed after morphine injection in various species[18–20] and was attributed to central effects of the drug[21], mediated by the adrenergic nervous system[22], but no studies of the acute effects of morphine on islet hormone release have ever been reported. The possibility that direct stimulation of glucagon release may contribute to hyperglycaemia, especially in states of insulin deficiency such as diabetes mellitus, should be considered.

Finally, it should be noted that in these studies significant suppression of SLI always preceded significant changes in insulin and glucagon release. This raises the possibility that a primary event in opiate action upon the islets is suppression of endogenous somatostatin and release of insulin and glucagon from its inhibitory influence.

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Induction of ornithine decarboxylase by nerve growth factor dissociated from effects on survival and neurite outgrowth

SEVERAL eukaryotic cell types respond to a variety of hormones or growth-promoting agents by a rapid induction of ornithine decarboxylase (ODC) activity (for reviews see refs 1, 2). This enzyme[2] and the polyamines whose synthesis it catalyses[1] have been suggested in turn to play a part in mediating subsequent cellular responses to such hormone or replication factors. Among the agents shown to affect ODC activity is nerve growth factor (NGF), a protein which affects the differentiation and survival of sympathetic and responsive sensory neurones[3]. MacDonnell *et al.*[4] have demonstrated that treatment of rat superior cervical ganglia with NGF causes a rapid (maximal by 6–7 h), transcription-dependent increase in ODC activity. NGF treatment has also been reported to increase ODC activity in rat brain[5]. Such findings have led to the suggestion that ODC may mediate other responses of neurones to NGF[4]. We show here, however, that suppression of ODC activity and of its induction do not interfere with several other responses to NGF such as promotion of neurite outgrowth, maintenance of survival and increase in cell size. Such findings may be relevant to the possible role of ODC induction in other differentiating systems.

One particularly useful model system for studying the mechanism of action of NGF is a clonal line (designated PC12) of rat adrenal phaeochromocytoma cells[6]. In serum-containing medium and in the absence of NGF, such cells display several differentiated properties associated with adrenal chromaffin cells[6,7]. When treated with nanogram levels of NGF, however, PC12 cells cease replication and acquire certain features of sympathetic neurones, including the formation of extensive neurites[6] (Fig. 3) and electrical excitability[6]. One of the advantages of the PC12 line is that it may be used to study the initial

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events whereby NGF affects target cells (normal responsive neurones, in contrast, have already been exposed to the factor *in vivo* before experimental intervention). Moreover, PC12 cultures maintained with serum can be used as adequate non-NGF-treated controls (normal sympathetic neurones, in contrast, require NGF for survival[9] and hence, NGF-untreated material may be dying and unsuitable as controls).

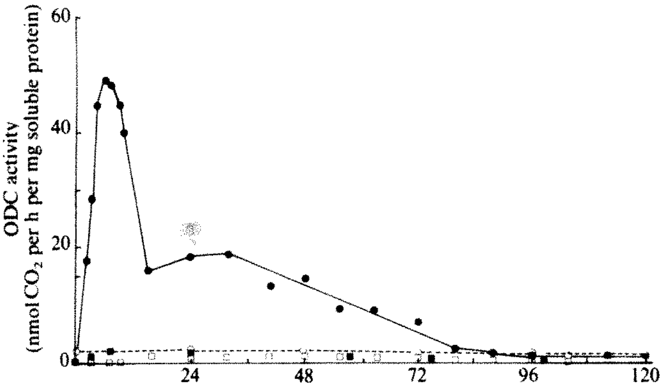

Fig. 1 Specific activity of ornithine decarboxylase in PC12 cultures after various times of treatment as indicated. PC12 cells (~250 generations after their isolation) were cultured on collagen-coated 35-mm tissue culture dishes (Falcon) as described previously[6,7], in 1.5 ml of medium. Because the introduction of fresh complete growth medium (85% RPMI 1640, 10% horse serum and 5% fetal calf serum)[6] to cultures itself caused a 15-fold induction of ODC activity within 6 h, the experiments shown here used cultures maintained in medium containing 1% horse serum. The latter medium did not induce ODC activity and hence long-term experiments (in which medium was changed every other day) could be carried out with a stable baseline. Although growth in such medium was slowed (doubling time ~6 d), neither cell viability nor responsiveness to NGF were affected. (It may also be noted that qualitatively identical results to those shown here were also obtained with cultures maintained in complete growth medium.) Cultures treated with 5-hexyne-1,4-diamine or 1,3-diaminopropane (Aldrich) were preincubated with these compounds for 14 and 1 h, respectively, before addition of NGF. Mouse salivary gland 2.5S NGF[10] was added in 5 μl of culture medium to a final concentration of 50 ng ml^{-1}. Addition of the same volume of culture medium containing the diluted buffer (0.1 M sodium acetate, *p*H 5.5) in which the stock NGF (0.5 mg ml^{-1}) was dissolved had no effect on ODC activity. After various times of treatment, cultures were collected by scraping the cells into 400 μl of assay buffer (see below). The cells were broken by sonication, and the sonicates quick-frozen on dry ice and stored at −20 °C (<7 d) until assayed. Before assay, samples were thawed and centrifuged for 10 min at 12,000 *g* in a Brinkmann microcentrifuge. ODC activity was measured in the supernatant fraction by minor modifications of the assay described by Pegg and Williams-Ashman[11]. Assay buffer was 50 mM Tris-Cl, *p*H 7.4, containing mM sodium EDTA, 5 mM dithiothreitol, 0.1 mM pyridoxal phosphate and 1 mM D,L-ornithine. For each assay, 300 μl of cell supernatant (or of buffer for blanks), 150 μl of assay buffer and 50 μl of assay buffer containing 0.45 μCi of [1-^{14}C]-D,L-ornithine (specific activity 53 mCi $mmol^{-1}$; New England Nuclear) were mixed and incubated for 1 h at 37 °C in a disposable 17 × 100-mm polystyrene tube (Falcon) with a centre well suspended from a rubber stopper (Kontes Glass). Released ${}^{14}CO_2$ was trapped in 100 μl of NCS solubilizer (Amersham) absorbed on a piece of filter paper in the centre well. Assays were terminated by injecting 0.5 ml of 50% trichloroacetic acid into the tube through the stopper. After overnight incubation, the trapped ${}^{14}CO_2$ was counted using Instagel scintillant (New England Nuclear). Assays were linear for the range of protein used (60–300 μg per sample). Specific activities are based on protein concentrations determined by the method of McGuire *et al.*[12]. Values are averages of determinations on duplicate cultures. ○, Untreated; ●, NGF; □, NGF + 1 mM 1,3-diaminopropane; ■, NGF + 0.1 mM 5-hexyne-1,4-diamine.

Figure 1 shows the results of an experiment in which ODC activity was measured in PC12 cultures at various times of treatment with or without 50 ng per ml 2.5S mouse submaxillary gland NGF[10]. In NGF-containing cultures, ODC activity underwent a rapid increase which was maximal within 5–8 h of treatment. A smaller secondary increase was also present during the second day of exposure. For 12 separate experiments, the ODC activities (±s.e.m.) in cultures treated with or without NGF for 6 h were 41.0 ± 3.1 and 2.2 ± 0.5 nmol CO_2 released per h per mg protein, respectively. This rapid NGF-dependent increase in ODC activity seemed to require RNA transcription, as it was completely blocked (to levels of <0.9 nmol CO_2 released per h per mg protein) by exposure to the RNA synthesis inhibitors actinomycin-D (1 μg ml^{-1}) or camptothecin (10 μg ml^{-1}).

The effect of NGF on ODC activity was not generalised to all cell types. Exposure to NGF for 6 h did not alter the ODC activities in cultures of two cell lines (3T3 fibroblasts[13] or C_6 glioma cells[14]) which have no known biological response to the factor. The mean ($n = 3$) ODC activity ±s.e.m. (in nmol CO_2 formed per hr per mg protein) in 3T3 cultures was 20.5 ± 0.7 (no NGF) and 21.0 ± 1.2 (1,000 ng ml^{-1} NGF) and in C_6 cultures 5.3 ± 0.7 (no NGF) and 4.8 ± 0.2 (1,000 ng ml^{-1} NGF).

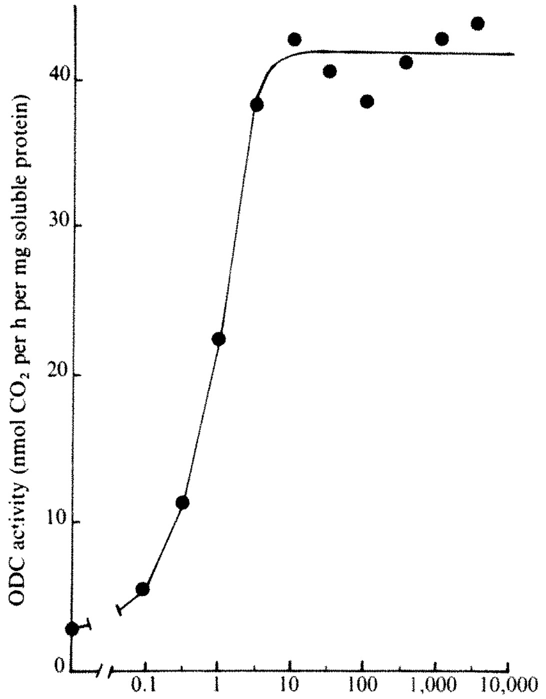

Fig. 2 ODC activity in sister PC12 cultures 6 h after exposure to the indicated concentration of NGF. Cultures were maintained as in Fig. 1 and assays were carried out as described in the legend to Fig. 1. Values are averages of determinations on duplicate cultures.

Figure 2 shows a dose–response relationship (after 6 h of treatment) between NGF concentration and ODC activity in PC12 cultures. Stimulation of activity was detectable at the lowest NGF concentration tested (0.1 ng ml^{-1}) and was maximal at 3–10 ng ml^{-1} (0.1–0.3 nM) NGF. The latter concentrations correspond to those required for stimulation of neurite outgrowth from PC12 cells[6,15] and are similar to the dissociation constant reported for NGF with membrane receptors on mammalian sympathetic ganglia[16].

The finding that NGF treatment causes a rapid increase in ODC activity of responsive cells raises the question as to whether such an increase is required for other responses to the factor. To test this, two methods were used to suppress ODC activity in PC12 cells, treatment with 1 mM 1, 3–diaminopropane (DAP) or with 0.1 mM 5-hexyne-1,4-diamine (HDA). The former suppresses ODC activity by apparently causing a specific inhibition of synthesis of the enzyme[17,18], and the latter is a specific, irreversible inhibitor of ODC activity[19] that is effective within living cells (R. Rando, personal communication). As shown in Fig. 1, treatment of PC12 cultures with either compound totally blocked the effect of NGF on ODC activity. At 6 h after addition of NGF, ODC activities in DAP- and HDA-treated cultures were 1 and 4%, respectively, of those in non-inhibited NGF-treated cultures. Nevertheless, treatment with these inhibitors did not affect either cell viability or the ability of NGF to promote neurite outgrowth. Figure 3 illustrates the morphological response of PC12 cells to NGF with or without continuous suppression of ODC activity.

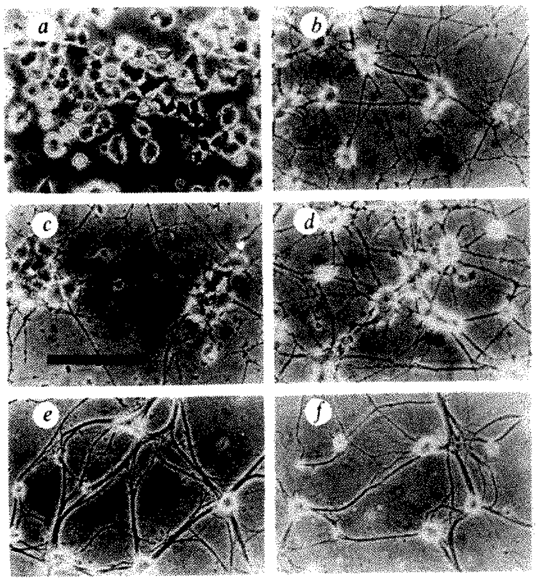

Fig. 3 Phase-contrast micrographs of PC12 cells maintained in culture conditions as described in Fig. 1 and treated for 6 d with (A) no NGF, (B) NGF, (C) NGF plus 1 mM 1,3-diaminopropane, or (D) NGF plus 0.1 mM 5-hexyne-1,4-diamine, and of dissociated cell cultures of 11-d embryonic chick sympathetic neurones maintained for 6 d (E) with NGF or (F) with NGF plus 1 mM 1,3-diaminopropane. Cultures of chick sympathetic neurones were prepared as previously described[20] except that the presence of non-neuronal cells was substantially reduced by treatment with 0.6 μM cytosine arabinoside on days 2–4 *in vitro*. Sympathetic neurones treated with 0.1 mM 5-hexyne-1,4-diamine resembled those shown in (E) and (F). Treatment with the ODC inhibitors in the absence of NGF promoted neither neurite outgrowth from PC12 cells nor survival of neurones. Bar, 100 μm.

Table 1 Effect of inhibitors of ODC activity on NGF-dependent survival of PC12 cells in serum-free medium

Addition to culture medium (RPMI 1640)	Cells per dish ($\times 10^{-4}$)
None	0.3 ± 0.2
0.1 mM 5-hexyne-1,4-diamine	0.3 ± 0.1
1.0 mM 1,3-diaminopropane	0.2 ± 0.1
50 ng ml^{-1} NGF	10.6 ± 1.7
50 ng ml^{-1} NGF + 0.1 mM 5-hexyne-1,4-diamine	12.2 ± 2.3
50 ng ml^{-1} NGF + 1.0 mM 1,3-diaminopropane	9.4 ± 1.5

PC12 cells (8×10^4) were plated in serum-free culture medium in the presence of the indicated compounds and maintained for 7 d. Cell number per dish was then determined as described elsewhere[6]. Values are means ± s.e.m. of four determinations.

As may be seen in Fig. 3, NGF treatment also causes an increase in the size of PC12 cell bodies[8]. This increase still occurred when induction of ODC activity was blocked. Measurement of cell body diameters (where diameter is defined as (length of short axis + length of long axis)/2) from photographs of cells gave mean ± s.e.m. ($n = 25$ in each case) values of 9.4 ± 0.2 μm for untreated cells, and 13.6 ± 0.5 μm, 13.9 ± 0.4 μm and 13.5 ± 0.4 μm, respectively, for cultures treated for 6 d with NGF, NGF + 1 mM DAP, and NGF + 0.1 mM HDA. Such results did not seem to be due merely to cell flattening, as similar changes in size were also apparent when the cells were detached from the dish and allowed to round up in suspension.

Another effect of NGF on PC12 cells is to promote their survival (as well as their morphological differentiation) in serum-free medium[15]. That is, if serum is withdrawn from PC12 cultures, the cells die. However, in the presence of NGF in serum-free medium, the cells survive and extend neurites. In the present experiments, NGF was found to induce ODC in serum-free conditions. The average ODC activities in duplicate cultures treated for 6 h with or without NGF in serum-free culture medium were 26.8 and 1.8 nmol CO_2 released per h per mg protein, respectively. However, as shown in Table 1, suppression of ODC activity in serum-free PC12 cultures by either DAP or HDA did not block the effect of NGF on cell survival.

Experiments were also carried out with dissociated cell cultures of 11-d chick embryo sympathetic neurones. These cells require NGF for their survival and neurite outgrowth[3,20]. Inclusion of either mM DAP or 0.1 mM HDA in NGF-containing culture medium at the time of plating of such cells did not have an apparent effect on their survival or neurite outgrowth (see Fig. 3) even though ODC activity (measured at 6 d after plating) was suppressed to only 2 and 12%, respectively, of that in control cultures (38.4 pmol CO_2 released per h per mg protein).

Our findings strongly suggest that although NGF can induce ODC activity in responsive cells, neither increased levels of the enzyme molecule nor of its enzymatic activity are required for NGF-dependent survival or stimulation of neurite outgrowth. This conclusion raises several alternatives concerning the role of ODC induction in the mechanism of action of NGF. One is that induction of ODC is causally related to other effects of NGF which were not tested here. Another possibility is that ODC induction bears no causal relationship to other actions of NGF and that it is itself a 'dead end' or terminal consequence of other events related to the interaction of NGF with its target cells. The latter possibility raises the issue of the relevance of ODC induction to the mechanism of action of other agents[1,2] which affect cell differentiation.

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Cyclic-AMP-induced differentiation in neuroblastoma is independent of cell division rate

CLONED, cultured mouse neuroblastoma cells, when grown in conditions which restrict cell division, extend neurites[1–6] and increase the activity of neurotransmitter-related enzymes[7–9]. Treatments that can induce this differentiation include lowered serum concentrations[3], prostaglandins[4], phosphodiesterase inhibitors[5] and analogues of cyclic-3′,5′-AMP (cyclic-AMP)[6,10–11]. These treatments have been proposed to act by elevating intracellular cyclic-AMP levels, but because they also inhibit cell division it has been difficult to determine whether their effects are due directly to cyclic-AMP or indirectly to inhibition of cell division[12]. As neurite growth and enzyme elevation occur during the interphase portion of the cell cycle (and are retarded during cell division), cyclic-AMP may simply allow differentiation by stopping the cell cycle in interphase[3]. The butyryl analogues of cyclic-AMP, in particular, markedly restrict cell division (ref. 10; see also Fig. 1*a*). Because butyrate, which can dissociate from the dibutyryl analogue of cyclic-AMP, can itself markedly inhibit cell division we reasoned that other analogues may exist which are not inhibitors of cell division but which effectively mimic the action of cyclic-AMP. We report here that 8-bromo-cyclic-3′,5′-AMP (8Br-cyclic-AMP) effects biochemical and morphological changes in the neuroblastoma clone NBD-2 (ref. 9) without concomitant inhibition of cell division.

As shown in Fig. 1 *a*, 8Br-cyclic-AMP (up to 1 mM) does not inhibit the rate of cell division in this neuroblastoma culture but, in fact, below 0.3 mM produces an increase in the cell division rate (150–160% of control). In contrast, both dibutyryl-cyclic-AMP (Bt_2-cyclic-AMP) and Na butyrate inhibit cell division. At 1 mM, Bt_2-cyclic-AMP inhibits division ~50% and Na butyrate inhibits cell division almost completely. (0.2 mM Na butyrate produces an inhibition of cell division comparable to 1 mM Bt_2-cyclic-AMP.)

Table 1 Enzyme activities in the NBD-2 cells

Treatment	TH	CAT	AChE	Cell no. per culture*
	Product per h per 10^6 cells†			
	(pmol)	(pmol)	(μmol)	
Control	50±9	3.3±0.3	5±3	6.4±0.8
1 mM 8Br-cyclic-AMP	810±12	9.9±1.0	6±3	6.2±1.1
1 mM Bt_2-cyclic-AMP	760±10	10.2±0.5	120±11	2.9±0.3
0.2 mM butyrate	62±8	3.9±0.8	6±4	2.9±0.5

NBD-2 cells were plated at 0.4×10^6 cells per 60 mm diameter culture dish in 5 ml of F-12 medium supplemented with 10% newborn calf serum. Treatments were added after 24 h, and cells were collected for assay 48 h later. The enzyme assays used are described in detail elsewhere[9].

* Values are expressed in terms of 10^6 cells after 72 h in culture per 10^6 cells originally plated.

† All values represent the mean ±SEM of four determinations.

On the other hand, 8Br-cyclic-AMP and Bt_2-cyclic-AMP, but not Na butyrate, induce comparable increases in neurite extension (Fig. 1*b*). Thus, the cyclic-AMP moiety, as opposed to decreased cell division, seems to be responsible for the morphological changes in these cells. However, because the data in Fig. 1 are taken from a population of cells at a single time point, it is still possible that only some of the cells undergo neurite extension (and diminished cell division) while other cells increase their rate of division. For example, at low 8Br-cyclic-AMP concentrations (little effect on neurite extension) perhaps a small, more rapidly dividing subpopulation is being revealed, and as the 8Br-cyclic-AMP concentration is raised this effect becomes relatively balanced by another subpopulation of cells now undergoing neurite extenstion (and not dividing).

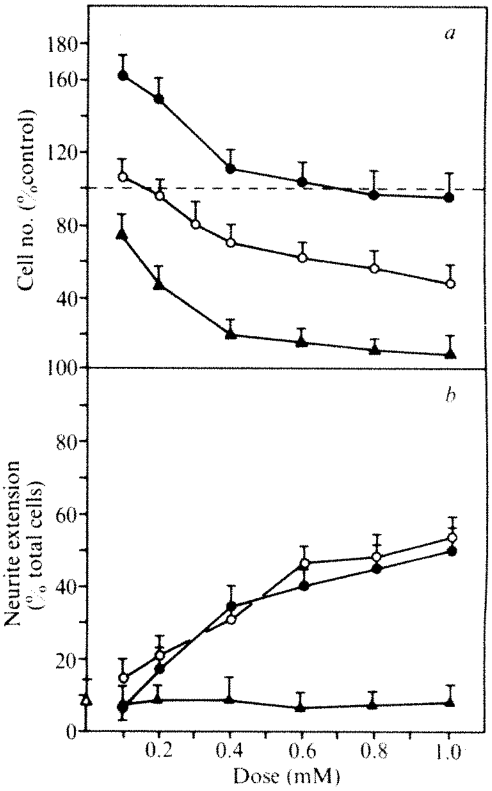

Fig. 1 Cell division and neurite extension in cultured NBD-2 neuroblastoma cells. NBD-2 neuroblastoma cells, isolated as previously described[9], were plated at 0.4×10^6 cells per 60-mm diameter culture dish in 5 ml of F-12 medium supplemented with 10% (v/v) GG-free newborn calf serum. Treatments were begun 24 h later and continued for 48 h. Drugs and media were changed every 24 h. *a*, Cell numbers were determined by haemocytometry on the basis of trypan blue exclusion[9]. More than 99% of the cells were trypan blue excluding. Values represent the mean ±SEM of four separate experiments in which duplicates were run for each determination. *b*, The number of cells extending neurites was determined in each sample by counting the number of cells (out of 200) with processes greater than twice the length of the cell body. Values represent the mean ±SEM of four separate experiments as in *a*. △, Control; ○, Bt_2-cyclic-AMP; ●, 8Br-cyclic-AMP; ▲, butyrate.

Examination of 8Br-cyclic-AMP-treated neuroblastoma cells with time-lapse cinematography (3 d) revealed, however, that every cell within the microscopic field underwent both neurite extension and division during each 24-h period. Figure 2 is taken from one time-lapse film and shows that the 8Br-cyclic-AMP-treated cells exhibit both neurite extension and cell division. The cells spent the majority of time in a differentiated state (Fig. 2 *a,d,j*) and, with rare exception, were de-differentiated only immediately before and after division. For example, the average

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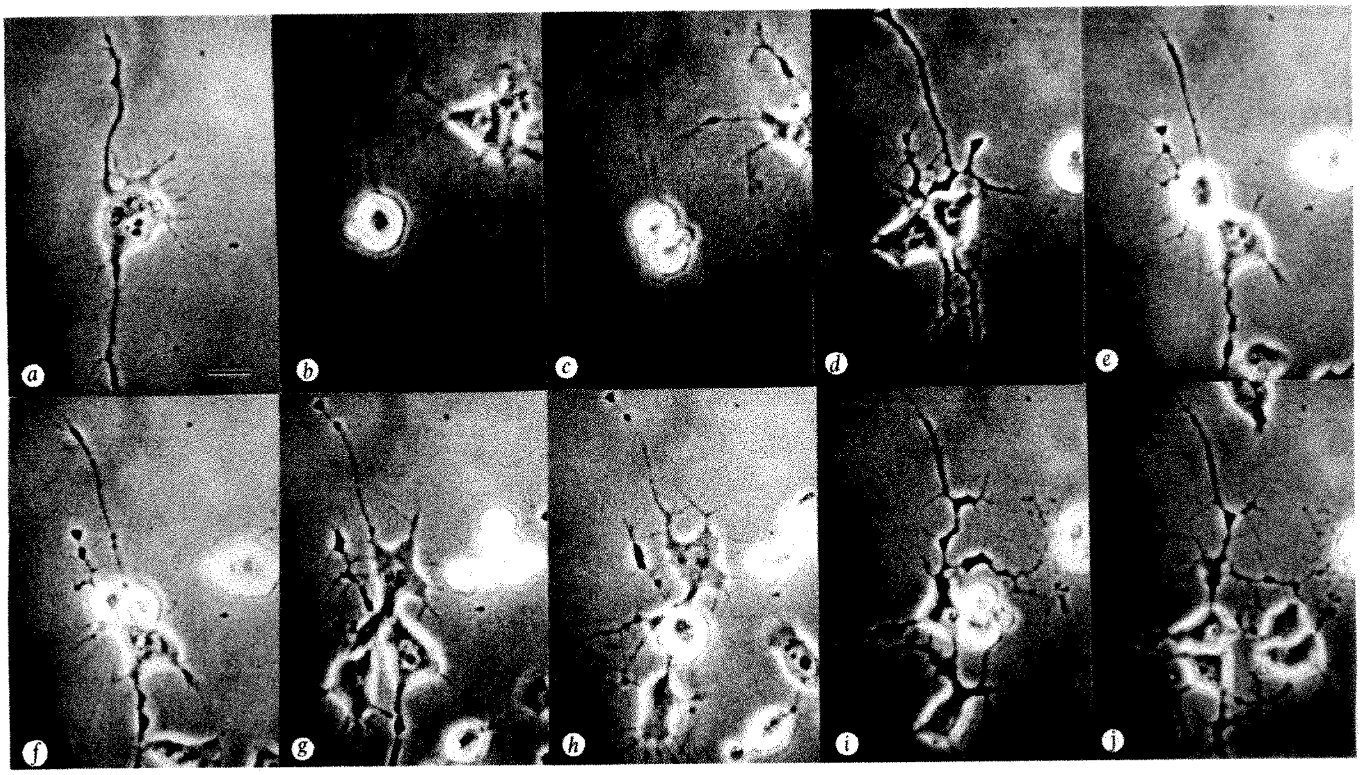

Fig. 2 Differentiation and division in NBD-2 cells treated with 8Br-cyclic-AMP. NBD-2 cells were plated at 0.1×10^6 cells per 2 ml F-12 medium supplemented with 10% newborn calf serum in Rose chambers and gassed with 5% CO_2 95% air. After 24 h, the cells were treated with 1 mM 8Br-cyclic-AMP and time-lapse cinematography was begun ~10 h later. For the first 15 h (*a*) the cell remained in a differentiated state. It withdrew its processes and remained rounded up (*b*) for 40 min whereupon it divided (*c*). After 50 min both cells exhibited processes at least twice the diameter of the cell body (*d*). The cells remained in this differentiated state for 18 h until one of the cells de-differentiated and divided over a 54-min period (*e*, *f*). The newly divided cells differentiated (*g*, 100 min) and the second cell subsequently de-differentiated (*h*, 2 h) and divided (*i*, 40 min). The cells differentiated (*j*) and all four cells remained differentiated for the duration of this particular film (>15 h). The photography was taken on a Zeiss RA microscope with phase contrast optics in a hot room at 37 °C. A Sage series 500 cinephotomicrographic apparatus with a Bolex H167 16-mm movie camera was used with 0.5 s exposures at 1/s. The calibration bar represents 10 μm.

time in a de-differentiated state was 77 ± 12 min ($n = 10$ randomly selected divisions).

Stopped-frame analysis at 4-h intervals showed that 40–60% of the treated cells exhibited neurite extension at any given time, in agreement with Fig. 1*b*. Thus, as this division rate is comparable to untreated cells[9] and as growth curves of 8Br-cyclic-AMP-treated and untreated cells are identical (data not shown), the increase in neurite extension cannot be attributed to a decrease in division rate *per se*.

Concomitant with the changes in cell morphology produced by the two cyclic-AMP analogues, tyrosine hydroxylase (TH, EC 1.14.16.2) and, to a lesser extent, choline acetyltransferase (CAT, EC 2.3.1.6) activities are increased (Table 1). The fact that these changes in enzyme levels are induced by both 8Br-cyclic-AMP and Bt_2-cyclic-AMP (but not Na butyrate at a concentration that produces an inhibition of cell division equivalent to 1 mM Bt_2-cyclic-AMP) implies that the cyclic-AMP moiety of these compounds is also capable of modifying the biochemisty of the cultured neuroblastoma cells in a manner independent of an effect on cell division rate. Interestingly, this was not the case for a third enzyme examined. 1 mM Bt_2–cyclic-AMP, but not 0.2 mH Na butyrate or 1 mM 8Br-cyclic-AMP, dramatically elevated the activity of acetylcholinesterase (AChE, EC 3.1.1.7) (Table 1). Although the elevation of this enzyme is often associated with decreased cell division[7,13–15], the lack of effect of Na butyrate (0.2 mM, matched for effects on cell division) argues against a simple cause. However, as higher Na butyrate concentrations do induce comparable increases in AChE activity (not shown), some butyrate (either attached or dissociated from cyclic-AMP) or butyrate-cyclic-AMP interaction may be responsible for this effect.

These results, then, strongly support the contention that cyclic-AMP can influence neuroblastoma morphology and biochemistry in a manner independent of changes in cell division rate. Further, 8Br-cyclic-AMP seems to be better suited than at least the dibutyryl analogue of cyclic-AMP for studies in which effects on cell division may complicate interpretation of the results.

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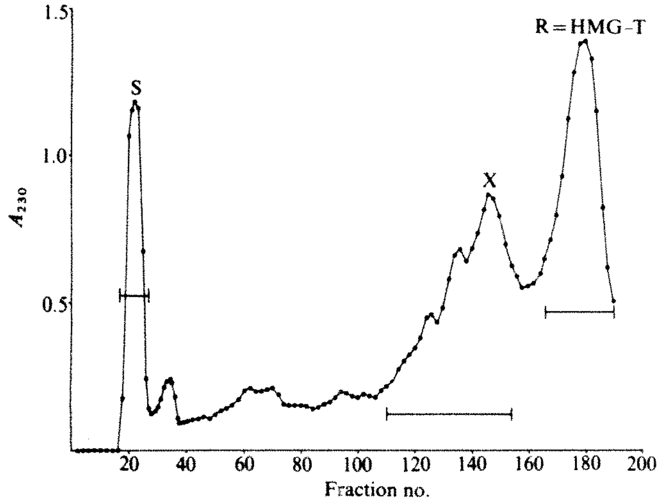
Free ubiquitin is a non-histone protein of trout testis chromatin

A NON-HISTONE chromosomal protein extracted from trout testis chromatin by 0.35 M salt, protein S, (ref. 1) has now been purified to homogeneity and its primary structure determined (Fig. 3) and shown to be identical with that of the thymus protein, ubiquitin[2-4] (originally called thymopoietin) and sequenced by Schlesinger *et al.*[5]. Ubiquitin was previously reported in chromatin by Goldknopf *et al.*[6] as a component of protein A-24 in which it is covalently bound by the α-carboxyl group of its C-terminal glycine to the ε-amino group of lysine 119 of histone H2A in an isopeptide linkage[7]. Goldknopf and Busch[8] have reported that protein A-24 is a component of the core nucleosomes of rat liver chromatin being present in quantities sufficient for it to be linked to H2A in 10–20% of the total nucleosomes. The present studies indicate clearly that ubiquitin can also occur as a free polypeptide in trout testis chromatin and is readily extracted by 0.35 M NaCl. Moreover, free trout testis ubiquitin (protein S) is rapidly released together with HMG-T (ref. 9) (and small amounts of Hl) during limited micrococcal nuclease digestion of trout testis chromatin and is probably also located in the linker regions of the chromatin. DNase I digestion does not release free ubiquitin preferentially[10]. In view of the pronounced biological effects of free ubiquitin on membrane adenyl cyclase of immature thymocytes[3,4] and the ability of ubiquitin to promote the differentiation of these cells into mature T cells[3,4], its occurrence in chromatin raises the possibility of a role of ubiquitin in cell differentiation and differential gene expression.

Trout testis ubiquitin (protein S) was isolated from washed chromatin by the method described by Goodwin *et al.*[11] for preparation of calf thymus HMG-proteins and was purified by chromatography on a BioRex 70 cation exchanger[1] (Fig. 1); ubiquitin was eluted as a sharp peak at the passthrough volume of the column and on electrophoresis either in starch[12] (Fig. 2*a*), Triton-polyacrylamide[13] gels (Fig. 2*b*) or SDS-polyacrylamide gels[14] (Fig. 2*c*), it migrated as a single component with a minor slower running contaminant. The mobility of trout testis ubiquitin is identical with that of authentic calf thymus ubiquitin (Fig. 2*b*). Comparison with the mobility of the molecular weight protein markers on the SDS-polyacrylamide gel (Fig. 2*c*) gives molecular weights of 8,500 for both trout testis and calf thymus ubiquitin.

Fig. 1 Ion-exchange chromatography of trout testis HMG-proteins on a column (2 × 50 cm) of BioRex 70 (200–400 mesh) eluted with a linear gradient formed by mixing 500 ml of 2% with 500 ml of 11% guanidinium chloride[1]. The fractions containing the protein peak emerging at the pass-through volume of the column (S) were combined, exhaustively dialysed in membranes of Sepraphore III (MW cutoff 3,500) against distilled water and lyophilised. The yield of protein S was approximately 17 mg per kg trout testis.



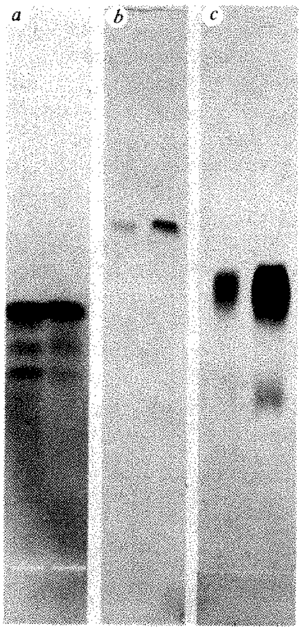


Fig. 2 Gel electrophoresis in three systems of trout testis ubiquitin (protein S) with markers of authentic calf thymus ubiquitin. *a*, Starch gel electrophoresis in aluminium lactate buffer[12]. *b*, Triton-polyacrylamide[13] gel electrophoresis (15% polyacrylamide, 0.22% Triton X-100, 5% acetic acid). *c*, SDS-polyacrylamide[14] gel electrophoresis (15% polyacrylamide, 1% SDS, Tris-glycine, *p*H 6.8). Molecular weight markers of insulin (6,000), lysozyme (14,300) and cytochrome *c* (12,000) were included in order to estimate the MW of the ubiquitins. The ubiquitin bands in (*c*) were excised, hydrolysed[15] and their amino acid compositions determined using a Beckman Model 121 M analyser equipped with a Systems AA integrator. Amino acid compositions were reduced to the most likely stoichiometry using the least squares fit program of Osawa and Tanaka[24].

Amino acid analyses show that trout testis and calf thymus ubiquitin are identical in composition within experimental error. In these analyses, the trout testis ubiquitin major component was eluted from the SDS-polyacrylamide gel and hydrolysed[15]. We have also carried out a completely independent amino acid sequence analysis of trout testis ubiquitin by automatic Edman degradation of the intact protein as well as a series of peptides obtained by trypsin cleavage after protection of the lysyl residues by maleylation[5], by chymotryptic cleavage, by cleavage with staphylococcal protease[16], by chemical cleavage at aspartyl residues in 0.2 M acetic acid[17] and by chemical cleavage at the single tyrosyl residue at position 59 with *N*-bromosuccinimide. The single methionyl residue is amino-terminal and it was found that cleavage with cyanogen bromide yielded free homoserine lactone and a fragment (residues 2–74) indistinguishable from the intact protein by gel electrophoresis.

The only area of uncertainty in the sequence is between residues 68 and 71, as it has not yet been possible to recover from trout testis ubiquitin a peptide containing the histidyl residue located at position 68 of calf thymus ubiquitin. A summary of the peptides leading to the deduction of the complete sequence of trout testis ubiquitin is given in Fig. 3. Details of the sequence analysis will be published elsewhere. This sequence is identical to that described for calf thymus ubiquitin by Schlesinger *et al.*[5] with the possible exception of residues 68–71. Our independent determination of the complete sequence of ubiquitin from a salmonid fish testis and the demonstration of the identity of at least 71 of the 74 positions with ubiquitin from calf thymus shows that the structure of ubiquitin is probably as highly conserved during evolution as that of the arginine-rich histones H3 and H4. A limited

N-terminal sequence is available for a plant ubiquitin (from celery)[4] and shows that six of the eight residues in this region are identical but the sequence is too limited to make a conclusion about the evolutionary conservation of ubiquitin in the plant kingdom. Note that a radioimmunoassay developed by Goldstein *et al.*[4] for ubiquitin detects the protein in vertebrates, invertebrates, plants, fungi and a variety of bacteria so it is likely that there has been only limited divergence in the major antigenic determinants during evolution.

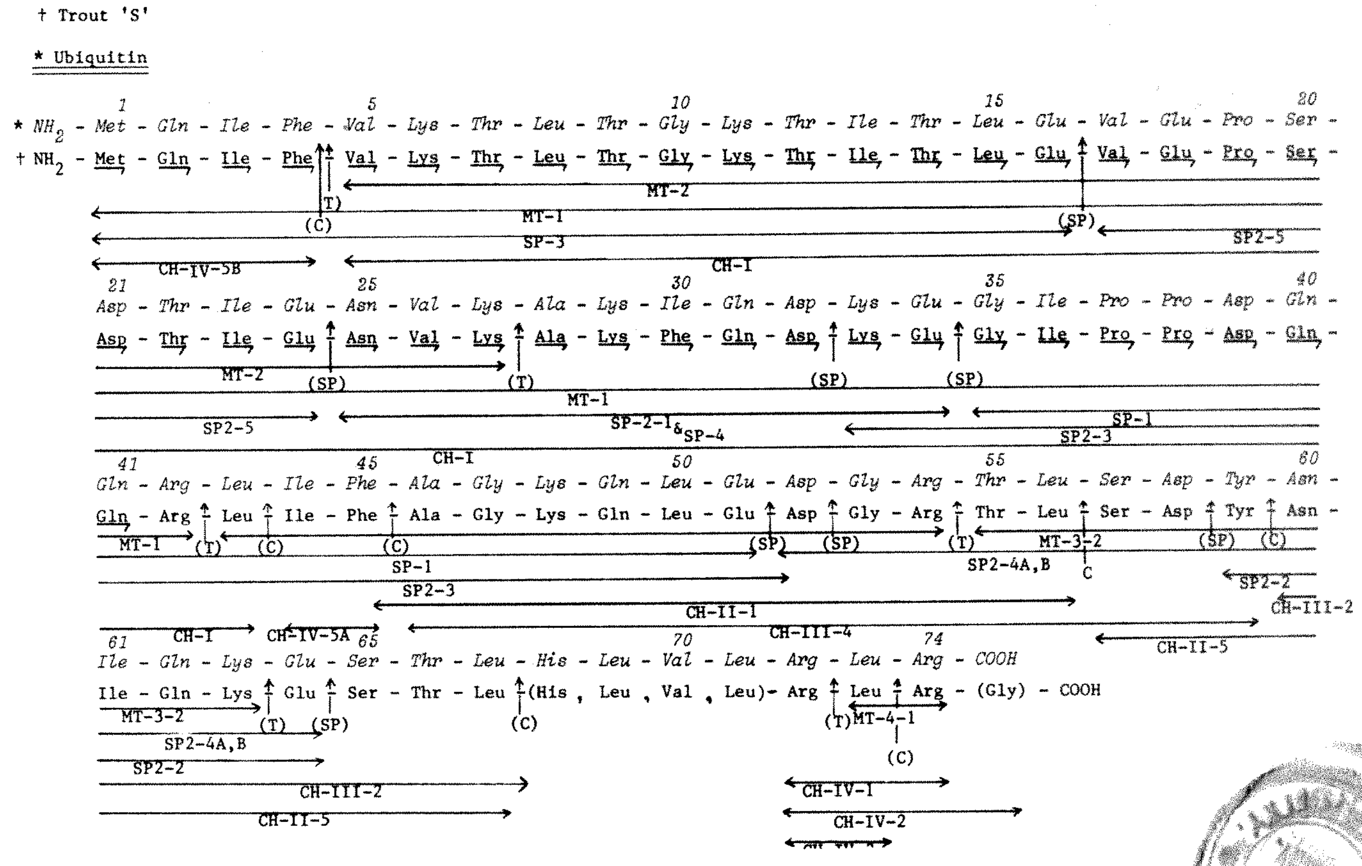
Table 1 Amino acid composition of calf thymus ubiquitin and trout testis protein S

	Calf thymus ubiquitin		Trout testis protein S	
	Moles %	Residues	Moles %	Residues
Asp	9.81	(7)	9.05	(7)
Thr	9.31	(7)	8.77	(7)
Ser	4.23	(3)	4.34	(3)
Glu	15.57	(12)	15.97	(12)
Pro	3.72	(3)	3.97	(3)
Gly	6.77	(5)	6.56	(5)
Ala	2.71	(2)	2.59	(2)
Val	5.41	(4)	5.08	(4)
Met	0.85	(1)	1.20	(1)
Ile	8.80	(7)	9.79	(7)
Leu	11.68	(9)	12.56	(9)
Tyr	1.18	(1)	1.39	(1)
Phe	2.54	(2)	2.59	(2)
His	1.35	(1)	1.29	(1)
Lys	9.64	(7)	9.79	(7)
Arg	5.25	(4)	5.08	(4)
	Total	75	Total	75

Ubiquitin has been noted previously as a constituent of protein A-24 of calf thymus or rat liver nuclei[6,8] in which it is covalently bound by an iso-peptide linkage[7] from a C-terminal glycine residue to the ε-amino group of lysine 119 of histone H2A to produce a branched protein. The identification of one 'arm' of this protein with calf thymus ubiquitin was made when Hunt and Dayhoff[18] noted that a sequence of 40 residues at the N-terminus of protein A-24 was identical with calf thymus ubiquitin (the H2A arm is blocked to Edman degradation in the sequencer by its N-terminal acetyl group). The molecular weight of A-24 has been reported as 27,000 by SDS-polyacrylamide electrophoresis[6] but there is a discrepancy between this value and the sum of the molecular weights of ubiquitin (8,451) and H2A (14,000). It is possible that A-24 may migrate anomalously in SDS-polyacrylamide gels, as both components, ubiquitin and H2A have strongly hydrophobic regions and could bind additional detergent molecules. The second discrepancy is that Schlesinger *et al.*[5] originally reported that the C-terminal residue of calf thymus ubiquitin was arginine, whereas the peptide containing the isopeptide linkage isolated by Goldknopf and Busch[7] was reported as comprising a sequence Gly-Gly, presumably at the C-terminus of the ubiquitin arm. In our sequence studies of trout testis ubiquitin we have noted heterogeneity at the C-terminus, 50% of molecules are terminated by arginine (Fig. 3, peptide CH-IV-1) and 50% by glycine (Fig. 3, peptide CH-IV-2) but we have not seen evidence of a Gly-Gly sequence at the C-terminus. Further work is required to establish whether protein A-24 contains sequences additional to the reported sequence of calf thymus and trout testis ubiquitin and which might account for the apparent extra size of calf thymus A-24. Application of the method of Goldknopf *et al.*[6] to trout testis chromatin in our hands has not, so far, yielded a protein corresponding to A-24.

When trout testis nuclei were digested to a limited extent with micrococcal nuclease in conditions which lead to the rapid digestion of the 'linker' regions of chromatin (that is, between nucleosomes)[19-23], we observed that ubiquitin was released into

Fig. 3 Alignment of peptides from trout testis ubiquitin and comparison with the amino acid sequence of calf thymus ubiquitin[5]. MT, peptides from tryptic digestion of maleylated ubiquitin; CH, chymotryptic peptides; SP, staphylococcal protease peptides. Automated sequence determination was performed on purified peptides or intact ubiquitin on a Beckman 890C protein sequencer using a programme similar to that of Edman and Begg[25] but replacing the Quadrol buffer with the volatile DMAA buffer. The 2-anilino-5-thiazolinone derivatives of the amino acids from the sequencer were identified and quantitated by amino acid analysis following back hydrolysis in 47% hydriodic acid at 150° for 20 h.


† Trout 'S'
* Ubiquitin
* NH2 - Met - Gln - Ile - Phe - Val - Lys - Thr - Leu - Thr - Gly - Lys - Thr - Ile - Thr - Leu - Glu - Val - Glu - Pro - Ser -
† NH2 - Met - Gln - Ile - Phe - Val - Lys - Thr - Leu - Thr - Gly - Lys - Thr - Ile - Thr - Leu - Glu - Val - Glu - Pro - Ser -
MT-2
(T)
(C)
MT-1
(SP)
SP-3
SP2-5
CH-IV-5B
CH-I
Asp - Thr - Ile - Glu - Asn - Val - Lys - Ala - Lys - Ile - Gln - Asp - Lys - Glu - Gly - Ile - Pro - Pro - Asp - Gln -
Asp - Thr - Ile - Glu - Asn - Val - Lys - Ala - Lys - Phe - Gln - Asp - Lys - Glu - Gly - Ile - Pro - Pro - Asp - Gln -
MT-2
(SP)
(T)
(SP)
(SP)
MT-1
SP2-5
SP-2-1&SP-4
SP-1
SP2-3
CH-I
Gln - Arg - Leu - Ile - Phe - Ala - Gly - Lys - Gln - Leu - Glu - Asp - Gly - Arg - Thr - Leu - Ser - Asp - Tyr - Asn -
Gln - Arg - Leu - Ile - Phe - Ala - Gly - Lys - Gln - Leu - Glu - Asp - Gly - Arg - Thr - Leu - Ser - Asp - Tyr - Asn -
MT-1
(T)
(C)
(C)
(SP)
(SP)
(T)
MT-3-2
(SP)
(C)
SP-1
SP2-4A,B
C
SP2-3
SP2-2
CH-II-1
CH-III-2
CH-I
CH-IV-5A
CH-III-4
CH-II-5
Ile - Gln - Lys - Glu - Ser - Thr - Leu - His - Leu - Val - Leu - Arg - Leu - Arg - COOH
Ile - Gln - Lys - Glu - Ser - Thr - Leu - (His , Leu , Val , Leu) - Arg - Leu - Arg - (Gly) - COOH
MT-3-2
(T)
(SP)
(C)
(T)
MT-4-1
(C)
SP2-4A,B
SP2-2
CH-III-2
CH-II-5
CH-IV-1
CH-IV-2
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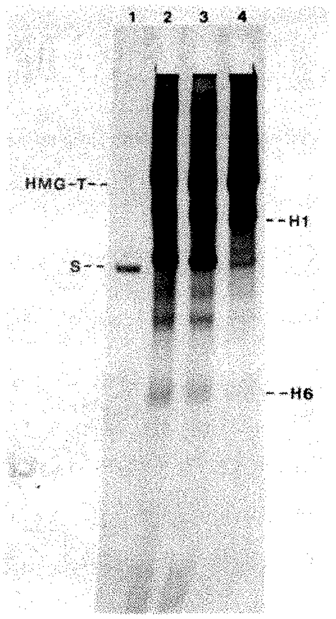

Fig. 4 Electrophoretic analysis of acid soluble proteins solubilised by micrococcal nuclease from trout testis nuclei. Micrococcal nuclease digestion of trout testis nuclei: trout testis nuclei were prepared as described previously[10]. The nuclear pellet was washed twice with RSB buffer (0.01 M Tris-HCl, *p*H 7.4, 0.01 M NaCl, 3 mM $MgCl_2$). Microscopic examination of the nuclear suspension revealed nuclei free of cytoplasmic tabs. Nuclei were resuspended in RSB buffer containing 1 mM $CaCl_2$ at a concentration of 10 mg ml^{-1} (200 A_{260}) of DNA and incubated with micrococcal nuclease (Worthington, 250 units ml^{-1}) at 37°C and digested to the extent of 5–10% of the input DNA. The reaction was terminated by chilling the tubes on ice and immediate centrifugation for 5 min at 1,000 *g* in a Sorvall HB-4 rotor. The supernatant was designated S_1 and used together with the pellet for protein extraction. Acid extraction of proteins and gel electrophoresis: chromosomal proteins were extracted from S_1 and pellet fractions by incubation of that fraction with 0.2 M H_2SO_4 for 1 h at 4°C, as described previously[10]. Three batches of trout testis nuclei, collected at different stages of maturation were digested with micrococcal nuclease to the extent of 10–15% solubilisation of the input DNA, as previously described. The acid soluble proteins from the soluble supernatant were analysed in 12% polyacrylamide gels containing 0.22% Triton X-100. This gel system allows complete separation of the histones[13] and also of HMG-T, ubiquitin (S) and H6. Electrophoresis was for 21 h at 300 V at 4°C. The gel was stained with amido black for 15 h and destained with 5% acetic acid. Slot 1 contains a sample of purified trout testis ubiquitin. Slot 2 shows the proteins solubilised after 15% digestion of middle-late testis nuclei. Slot 3: proteins solubilised after 12% digestion of middle-late testis nuclei. Slot 4: proteins solubilised after 8% digestion of late testis nuclei.

the soluble fraction together with the bulk of the HMG-T. The electrophoretic pattern in a Triton-containing polyacrylamide gel of proteins solubilised by micrococcal nuclease is illustrated in Fig. 4. Slots 2, 3 and 4 contain proteins released by the nuclease from three different batches of nuclei prepared from trout testis at various stages of maturation. In all three cases, it is clear that ubiquitin or protein S is released by the nuclease. The identity of the ubiquitin band was confirmed by excision of the stained bands and then subsequent acid hydrolysis and amino acid composition analysis. Each of the bands in slots 2, 3 and 4 showed amino acid compositions identical with the marker ubiquitin band (slot 1). Examination by gel electrophoresis of the proteins remaining in the nuclear pellet following micrococcal digestion (data not shown) indicates that no detectable band of ubiquitin remains following the nuclease treatment suggesting that ubiquitin is preferentially associated with linker regions in chromatin. However, the fraction represented by ubiquitin of the total proteins released by the nuclease in the soluble fraction varies in different batches of nuclei. This finding is consistent with our previous observations that the major protein released by micrococcal nuclease, namely HMG-T, also changes in concentration as a function of testis development.

Our finding of a free polypeptide in the low salt extractable non-histone fraction of trout testis chromatin which is virtually identical in primary structure with calf thymus ubiquitin indicates that ubiquitin is a true chromosomal protein which is widely distributed. The extreme conservation of its structure during evolution suggests that like the arginine-rich histones H3 and H4, ubiquitin has a vital structural role in chromatin. The fact that it constitutes only 2–5% of the concentration of the individual nucleosomal core histones further suggests that ubiquitin can only be present in a small sub-set of the chromatin and, therefore, that the conserved structure in which it is located is different from the bulk of the chromatin in the now well-established nucleosomal core and linker structure (beads-on-a-string).

Our experiments with micrococcal nuclease suggest that ubiquitin is present in an easily digested domain of chromatin which contains as a major protein constituent, the non-histone protein HMG-T. Furthermore, we have shown recently that during limited micrococcal digestion of trout testis chromatin, linker regions containing HMG-T (and ubiquitin) are rapidly solubilised and concomitantly, a sub-set of nucleosome core particles containing another non-histone protein, H6, is also released[22,23]. The DNA sequences present in this sub-set of nucleosomes are enriched five to tenfold in sequences hybridising with testis RNA and this degree of enrichment of DNA sequences is also confirmed by DNA-driven hybridisations[22,23]. Thus, there seems to be clear evidence of the localisation of three non-histone proteins, ubiquitin, HMG-T and H6 in a domain of chromatin enriched in DNA sequences which can be transcribed into cellular RNA. It is possible that these proteins, either individually or as elements of a pattern could provide recognition signals which are necessary but not sufficient in themselves for transcription of designated regions of chromatin.

We thank Dr Gideon Goldstein (Ortho Pharmaceutical Co.) for a sample of calf thymus ubiquitin and Dr E. H. Peters (Protein Laboratory, Faculty of Medicine, University of Calgary) for analysis of the C-terminal residue of trout testis ubiquitin by hydrazinolysis. This work was supported by the MRC of Canada.

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BEATRIZ LEVY W.
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UNIVERSITY OF SALFORD

CHAIR IN ORTHOPAEDIC MECHANICS

Applications are invited for the Chair in Orthopaedic Mechanics in the Salford Orthopaedic Appliance Unit which is a Joint Body of the University and the Salford Area Health Authority (Teaching).

Candidates should be qualified in engineering or science and should desirably have had clinical experience in the field of orthopaedics.

The salary will be within the professorial range, the minimum of which will be £9,443 p.a. (under review).

Applications should be received by the Registrar, University of Salford, Salford M5 4WT (from whom further particulars may be obtained) by December 18, 1978.

Please quote reference number ME/343/N. 8370(A)

THE UNIVERSITY COLLEGE OF WALES ABERYSTWYTH

DEPARTMENT OF ZOOLOGY

Applications are invited for the post of

RESEARCH ASSISTANT

tenable for two years under an S.R.C. funded project, on the biology of a nematode parasite of larval *Simulium* (Diptera).

Salary within the range £3,384 to £3,883 per annum.

Application forms and further information can be obtained from the Secretary, Department of Zoology, The University College of Wales, Penglais, Aberystwyth, to whom applications, naming two referees, should be sent within three weeks of the appearance of this advertisement.

8354(A)

THE UNIVERSITY COLLEGE OF WALES ABERYSTWYTH

DEPARTMENT OF GEOGRAPHY

RESEARCH IN HYDROLOGY

Applications are invited for the post of RESEARCH ASSISTANT commencing January 1, 1979, to work on a three-year project to determine the role of soil pipe discharge in streamflow generation in an upland catchment. The work involves the design and supervision of an instrument network, hydrograph analysis and computer modelling.

Candidates with an M.Sc. in Hydrology or a similar qualification preferred. Salary: £3,189 per annum.

Further information obtainable from Dr J. A. Jones, Department of Geography, University College of Wales, Aberystwyth, to whom applications should be submitted as soon as possible. 8355(A)

The Queen's University of Belfast

LECTURESHIP IN HUMAN GENETICS

Applications are invited for a lectureship in the Department of Medical Genetics from April 1, 1979 or such other date as may be arranged. Experience in biology, genetics not necessarily in the human field, medical sciences or cytogenetics is highly desirable. Responsibilities include teaching of human genetics to medical undergraduates. Initial placing, which will depend on experience and qualifications, will be made on the scale, under review, for lecturers: £3,885 to £7,755 with contributory pension rights under the F.S.S.U. or U.S.S.

Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. Closing date: December 8, 1978. (Please quote Ref. 78/N).

8352(A)

SHEFFIELD CITY POLYTECHNIC

DEPARTMENT OF HOTEL AND CATERING STUDIES AND HOME ECONOMICS

TECHNICIAN (FOOD STUDIES) —T1 or T2 (Post No. 13)

The person appointed will be required to work in areas where all aspects of food preparation, processing and presentation are taught to catering students at advanced level and will be required to prepare food and equipment for student use. A qualification in Food Technology or an allied subject would be appropriate.

For an appointment at T2 level, applicants should hold H.N.C., Advanced City & Guilds or equivalent or for an appointment at T1 O.N.C. or equivalent.

Salary Scale: T1 £2,676 to £2,967; T2 £2,967 to £3,339, plus £312 per annum pay award plus qualification allowance.

Requests for an application form in writing only please to the Recruitment Section of the Personnel Department, Sheffield City Polytechnic (Dept. NA), Halfords House, Fitzalan Square, Sheffield S1 2BB. Completed forms should be returned by November 24, 1978. 8334(A)

Anglian Water Authority

QUALITY OFFICERS – 2 Posts
Ref: R2S13 & R2S43 Grade 4

£4,179 – £4,476 p.a.

inclusive of supplements

Applications are invited for the above two posts, initially based in Cambridge, with possible transfer to Bedford or Ely area offices at a later date in the case of post R2S43. Preference will be given to persons possessing an appropriate degree or the I.W.P.C. Diploma, with experience in the water quality field and a knowledge of geochemistry would be advantageous in the case of post R2S13.

Duties include development control, under the appropriate statutes, aspects of solid wastes disposal investigations and monitoring, the provision of technical information and various field surveys as appropriate, under the direction of the Quality Planning Officer and/or field inspections, investigations of pollutions and other matters, sampling surveys and the determination of consent conditions under the direction of the appropriate Area Quality Officer.

Water Industry conditions of service are applicable and Regular car allowances are currently payable.

Application forms may be obtained from the Divisional Scientist at the address below, telephone Cambridge 61561 (Miss Hobbs) to whom they should be returned by Monday 27th November, 1978:—

GREAT OUSE RIVER DIVISION
Great Ouse House, Clarendon Road
Cambridge CB2 2BL 8371(A)

UNIVERSITY OF SOUTH FLORIDA

8 NEW FACULTY POSITIONS FOR THE DEPARTMENT OF MARINE SCIENCE

The Department of Marine Science at the University of South Florida, St Petersburg, announces the establishment of up to 8, new faculty positions. Marine Science at USF has been designated by the Board of Regents for the State University System for special funding by the State Legislature for its development of a graduate Center of Excellence. The Florida Institute for Oceanography, also located in St Petersburg, provides research vessel use and equipment for oceanographers in the State University System.

Application from persons in the following oceanographic specialties are encouraged:

> Phytoplanktonology, Marine Animal Physiology, Fishery Biology, Microbiology, Organic Geochemistry, Physical Chemistry, Radiochemistry, Geotechnical Properties of Sediments, Carbonate Petrology/Sedimentation, and High Resolution Geophysics.

Rank and salary will depend upon professional experience and accomplishment. The minimum for a nine month appointment for Professor is $24,000; Associate Professor, $17,000; and Assistant Professor, $15,000. The positions are fully State supported and tenure earning. Duties will involve approximately 75% research and 25% teaching.

The new appointments may be made at any time prior to the 1979-80 academic year. Applicants must have the Ph.D. in fields appropriate to their specialties and promising records of research accomplishment. The closing date for the receipt of applications is January 1, 1979.

Applicants should send complete résumés with 3 references to Dr John C. Briggs, Department of Marine Science, University of South Florida, 830 First Street South, St Petersburg, Florida 33701. The University is an affirmative action equal opportunity institution.

8241(A)

Geologist

for research in seismo-stratigraphy

The Royal Dutch/Shell Exploration and Production Laboratory at Rijswijk, near The Hague, carries out research in the field of exploration for and production of oil and natural gas. This research is closely related to the exploration and production efforts of Shell operating companies. The work, which in many cases is carried out as a multidisciplinary team effort, concentrates on the development and introduction of new techniques and the search for practical solutions to problems.

In the Geophysics Department of this laboratory work goes on in the latest developments in seismology as applied to oil exploration. We have a vacancy in this department for a sedimentary geologist to carry out research on the stratigraphic interpretation of seismic data. This will include the recognition and mapping of depositional sequences as well as more detailed interpretation. Preference will be given to candidates who have completed a Ph.D. in geology. They must be familiar with mathematics and statistics and preferably have some experience in seismic interpretation.

We can offer training opportunities both within and outside your professional field. Working hours are flexible to suit personal requirements. You must be prepared to undertake assignments overseas if necessary.

Travelling and other reasonable expenses will be paid for interviews in London and The Hague. Financial assistance will be given for the removal of household and personal effects to The Netherlands.

If you are interested in this position please write, with full details, to:

Shell International Petroleum Company Limited, Recruitment Division PNEL/12 (N1), Shell Centre, London SE1 7NA.

Shell

8369(A)

THE UNIVERSITY OF LEEDS
ASTBURY DEPARTMENT OF BIOPHYSICS

Applications are invited for two temporary posts of

POSTDOCTORAL RESEARCH FELLOW

in a group carrying out structural studies of enzymes as part of a continuing programme of work on the enzymes of folate metabolism being carried out under the supervision of Professor A. C. T North and Dr A. J. Geddes with support from the Science Research Council and Yorkshire Cancer Research Campaign.

One post of for a

CRYSTALLOGRAPHER

preferably with experience in protein crystallography, to continue the determination of the structure of dihydrofolate reductase which has reached an advanced stage. The appointment at present available is for a fixed term of nine months.

The second post is for a worker with experience in

PROTEIN CHEMISTRY, EXPERIMENTAL PATHOLOGY

or related fields to be responsible for the extraction, purification and structural studies of enzymes from human tissue. This appointment is for a fixed term of three years.

Starting salary in the range £3.883 to £5,129 on the IA Scale for Research and Analogous Staff (£3,883 to £6,555) (under review) according to age, qualifications and experience.

Preliminary enquiries to Professor A. C. T. North (Telephone (0532) 31751, ext. 6130) will be welcomed.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 42/1/D. Closing date for applications November 30, 1978. 8301(A)

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

VACANCIES

TECHNICIAN GRADE 5

IMMUNOLOGICAL RESEARCH
Two Posts

Post 1 H.N.C./degree with experience in immunology and laboratory organisation essential. Work will include tissue culture and animal research in infectious diseases.

Post 2 H.N.C./degree with experience in immunology and perhaps tissue culture. To join a clinical research project on the evaluation of immunological parameters in patients.

TECHNICIAN GRADE 3

PHYSIOLOGY
One Post

To assist with laboratory research on respiratory physiology and disease, including the use of experimental animals, There may also be a requirement to assist with practical classes for medical students. Emoluments (under review) Technician 5 £3,461 to £3,995, Technician 3 £2,963 to £3,335. Four weeks annual holidays plus 13 other days. For further details and application forms write, stating in which post interested, to the Establishment Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. Closing date November 23. 8295(A)

UNIVERSITY OF NAIROBI— KENYA

Applications are invited for the post o

SENIOR LECTURER

in the
DEPARTMENT OF CHEMISTRY

Applicants should have qualificatio in Physical Chemistry. In addition, th applicants must have a Ph.D. plu several years' teaching and researc experience. The appointee will b required to undertake the academi leadership in both teaching and research at the undergraduate and postgraduate levels.

Salary: K£2,988 to K£3,984 p.a (K£1=£1.35 sterling). The Britis Government may provide salary supplementation of £4,770 p.a. (sterling) fo married appointee or £3,252 p.a (sterling) for single appointee (reviewed annually and normally free o all tax) and provide children's education allowances and holiday visi passages. Family passages; non-contributory medical scheme; S.S.S.F. o F.S.S.U.; housing allowance.

Detailed applications (2 copies) wit curriculum vitae and naming 3 referee to be sent to the Registrar (Recruitment and Training), University o Nairobi, P.O. Box 30197, Nairobi Kenya, by December 11, 1978.

Applicants resident in U.K. shoul also send one copy to Inter-Universit Council, 90/91 Tottenham Court Road London W1P 0DT. Further details ma be obtained from either address.

8382(A)

Food Scientist/Technologist

for work of national and international importance

The Composition Sections of the Food Science Division provide a wide range of Scientific advice to those Divisions of the Ministry with executive responsibility for legislation on food and drink, and also maintains a close liaison with other Government departments having an interest in food composition and safety.

This appointment calls for an experienced food scientist/technologist to join a team concerned with protein based foods. The work will involve collating data on the composition, preparation and processing of raw materials and interpreting such data as a basis for advice related to present and future UK, EEC and International legislation. Little practical work is required but members of the Section must develop and maintain a broad interest in food processing, composition and legislation.

Candidates, normally aged under 32, must have a good honours degree or equivalent in food science, chemistry, biochemistry or closely related subject, coupled with at least 4 years experience in the food industry or a relevant area of research. Ability to collect and interpret information, prepare critical reviews, and participate in committee work at national and international levels advantageous.

Appointment as Senior Scientific Officer (£5620-£7360) with starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 1 December 1978) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote ref: SB/35/AE.*

Ministry of Agriculture, Fisheries & Food

Centre for Overseas Pest Research Aquatic Biocides Research Group, London

Aquatic Biologist

■ Join small team testing the acute and chronic toxicity of pesticides on fish and other non-target organisms ■ Participate in planning laboratory studies ■ Assist with the long term planning of the work programme ■ Extended periods of field work in the Tropics possible.

Good hons degree in aquatic biology, ecology or related discipline Relevant experience preferred; at least 2 years required for HSO appointment Higher degree with research experience in the field advantageous Age normally under 30 Appointment as Higher Scientific Officer (£4560-£5910) or Scientific Officer (£3300-£4880) according to qualifications and experience Ref: SA/49/JA.

■ Application forms (for return by 1 December 1978) from Miss M Cole, Centre for Overseas Pest Research, College House, Wrights Lane, London W8 5SJ.

ALL THESE POSTS ARE OPEN TO MEN AND WOMEN.

8351(A)

Science Group
CIVIL SERVICE

BIRKBECK AND IMPERIAL COLLEGES
(University of London)
RESEARCH OFFICER IN INTERACTIVE COMPUTER GRAPHICS

Applications are invited for a three-ear post funded by the Science Research Council to work jointly with Professor D. M. Blow and Professor T. L. Blundell in the development of interactive computer graphics for tructure analysis of biological systems including proteins, drug molecules and macromolecule-water interactions.

The hardware includes an Evans and utherland Picture System II driven by PDP11/60 computer linked to an BM370/165 mainframe computer.

Applicants should have postgraduate xperience in computing, preferably with knowledge of mini-computers.

Salary according to qualifications and xperience, within Grade IA; entry point not exceeding £4,408 plus London Weighting, £450.

Applications to Professor T. L. Blundell, Department of Crystallography, Birkbeck College, Malet treet, London WC1E 7HX, telephone 1-580 6622. 8380(A)

NATIONAL COLLECTION OF TYPE CULTURES
MICROBIOLOGIST VACANCY
(Basic Grade)

Post for a science graduate in, preferably microbiology. The post includes maintenance of the bacterial strains of the Collection. About 3,500 strains of more than 300 species of medical and veterinary interest are kept, maintained in the freeze-dried state, and regularly checked for viability and characteristics. The National Collection of Type Cultures has extensive records and the graduate will also be involved in control of the supply of cultures. Opportunities for taxonomic research and identification of fresh isolates are available.

Salary according to age, qualifications and experience on the N.H.S. Whitley Council Scientific scale, plus London Weighting.

Applications to:- Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9. Tel: 01-205 7041. 8296(A)

ARC ANIMAL BREEDING RESEARCH ORGANISATION
Statistics and Computing Department

STATISTICIAN

Applications are invited for a Statistician to assist in the analysis of experimental data arising from research in various aspects of physiology and genetics of farm animals. The work will involve some computer programming and use of statistical program packages. Experience of computer programming in FORTRAN would be useful. The post is located at the Organisation's Field Laboratory at Roslin, near Edinburgh.

Applicants should have an honours degree with statistics as a main subject, or equivalent qualifications. Appointment will be as Scientific Officer (£2,839 to £4,415) with starting salary depending on qualifications and experience. Non-contributory superannuation.

Further information and application forms are available from the Secretary, ARC Animal Breeding Research Organisation, West Mains Road, Edinburgh EH9 3JQ. Closing date November 25, 1978. 8311(A)

OVERSEAS DEVELOPMENT

KNOW-HOW: vital to developing countries

Extension Agronomist Sudan

At Nuba Mountains Agricultural Production Corporation to assist with implementation of field programmes. Applicants should have University degree in Agriculture or related science, with at least 5 years' experience of crop production and extension work in developing country.

Appointment 3 years. Salary (UK taxable) in range £7000—£8500 pa plus tax-free overseas allowance in range £2080—£4555 pa (Ref 331).

The post is wholly financed by the British Government under Britain's programme of aid to the developing countries. In addition to basic salary and overseas allowances other benefits normally include paid leave, free family passages, children's education allowances and holiday visits, free accommodation and medical attention. Applicants should be citizens of the United Kingdom.

For full details and application form please apply, quoting reference stating post concerned, and giving details of age, qualifications and experience to:—

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London SW1E 5DH.

8303(A)

ODM

HELPING NATIONS HELP THEMSELVES

The University of British Columbia

DEPARTMENT OF CHEMISTRY

Applications are invited from candidates with teaching and research interest in any aspect of INORGANIC CHEMISTRY including BIOLOGICAL INORGANIC CHEMISTRY for an appointment at the Assistant Professorship level.

Applications consisting of a curriculum vitae, a list of publications and the names and addresses of three referees should be sent to the undersigned before December 31, 1978. This post is open to both male and female applicants.

Professor Charles A. McDowell
Department of Chemistry
University of British Columbia
2075 Wesbrook Mall
Vancouver, British Columbia
Canada V6T 1W5

8344(A)

UNIVERSITY OF OTAGO
Wellington Cancer and Medical Research Institute
Wellington Clinical School of Medicine
Wellington, New Zealand

RESEARCH OFFICER (MEDICAL OR NON-MEDICAL)

Applications are invited from medical or science graduates for the position of Research Officer. Science graduates should possess a higher qualification such as Ph.D. and medical graduates if not in possession of a higher qualification would normally be expected to enrol for a higher degree. Preference will be given to a person with previous research experience. The appointee will work in the field of atherogenesis and experimental pathology under the directorship of Professor W. E. Stehbens. Commencing salary according to qualifications and experience within the scale NZ$14,673 to $25,729 (Medical); NZ$11,894 to $18,380 (Science graduates).

Further particulars are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from the Registrar of the University, P.O. Box 56, Dunedin, New Zealand, or from the Director of the Institute (Professor W. E. Stehbens), Wellington Clinical School, Wellington Hospital, Wellington, New Zealand.

Applications close on **January 15, 1979.** 8363(A)

ENTOMOLOGIST
TSETSE RESEARCH
MINISTRY OF OVERSEAS DEVELOPMENT AND
UNIVERSITY OF BRISTOL

Applications are invited for appoint ment as an Entomologist at the Tsets Research Laboratory, Langford (1 miles south of Bristol). The laborator is maintained by the Ministry o Overseas Development in collaboratio with the University of Bristol. Th successful candidate will be required t undertake research into the biology o tsetse flies with special emphasis o the development of efficient method for their control. Some overseas trave may be involved. Candidates shoul have a Higher Degree and should ideall have had previous experience wit tsetse flies. Salary in the range £3,88 to £7,754 (scale under review) wit membership of the Universities Super annuation Scheme.

Applications, with curriculum vita and the names of two referees, shoul be sent by December 31, 1978 to:

The Director, Tsetse Researc Laboratory, Langford House, Lan ford, Bristol BS18 7DU. 8304(A)

HERIOT-WATT UNIVERSITY
DEPARTMENT OF MATHEMATICS

CHAIR OF MATHEMATICS

Applications are invited from suitably qualified candidates for a recently created Chair of Mathematics. Applicants should have acknowledged ability in both teaching and research. Current research interests in the Department are in both pure and applied mathematics. The appointment will be from October 1, 1979 or such earlier date as may be agreed.

Application forms and further particulars are available from the Secretary of the University, Chambers Street, Edinburgh EH1 1HX to whom completed application forms should be returned by December 8, 1978. Ref No. 3/78.

8323(A)

INVERTEBRATE PALEONTOLOGIST
UNIVERSITY OF CINCINNATI

A tenure-track faculty position i invertebrate paleontology will b available at the University o Cincinnati as of September 1, 197 We are seeking a person with demo strated research experience in inverte brate paleontology. The Ph.D. i required. Candidates should possess thorough knowledge of systematics an the ability to apply this knowledge t a wide range of problems such a functional morphology, paleoecolog biostratigraphy, or phylogeny. Teach ing duties will be at the undergraduat and graduate levels, and the perso will supervise both M.S. and Ph.D theses. The person will also be ex pected to maintain an active researc program and cooperate in the depar mental program in paleontology. Ran and salary are negotiable. Deadline f receipt of applications is Januarf 3 1979.

Applications should include: (1) brief statement of research interes and goals; (2) curriculum vitae an experience; (3) complete bibliograph and (4) *names* of three reference Applications should be submitted t

Dr David L. Meyer
Department of Geology
University of Cincinnati
Cincinnati, Ohio 45221.

The University of Cincinnati is a Equal Opportunity/Affirmative Actio Employer. **8320(A)**

High-Calibre Research Biochemist

Shell Research Limited are looking for a high-calibre Scientist to join the Fermentation and Microbiology Division of their Biosciences Laboratory at Sittingbourne, Kent.

You will join a multi-disciplinary team engaged in research on microbial extracellular polysaccharides. The work will involve studies on the biochemistry of microbial exopolysaccharide formation and on the physiology of the micro-organisms.

We would like to hear from people with either a Ph.D. or a good honours degree in an appropriate subject and with research experience in a relevant area of biochemistry. Knowledge of fermentation techniques would be advantageous but not essential. The ability to think and act innovatively will be considered important.

There is scope and opportunity for promotion and you will be expected to display the qualities needed to reach more senior positions. We will offer a competitive salary and assistance will be given with relocation expenses where appropriate. You will be able to join our pension fund, enjoy flexible working hours and work in well-equipped laboratories which are situated on a 450 acre farmland site. There are also excellent facilities for social and sports activities. Please write giving all the relevant details about yourself or telephone for an application form to:
Shell Research Limited, Recruitment Division (N1) PNEL/11, Shell Centre,
London SE1 7NA. Telephone: 01-934 2948. 8300(A)

Shell

IMPERIAL COLLEGE RESEARCH FELLOW

Postdoctoral Agricultural Scientist required for key post in research team working on the applications of trace element surveys to agriculture. Applicant should hold good degree in agriculture or soil science and have some experience of geology. Starting salary according to age and experience in the range £4,359 to £5,847 (including London Allowance) with U.S.S.

Applications to Dr I. Thornton, Applied Geochemistry Research Group, Department of Geology, Imperial College, London SW7 2BP.
8293(A)

UNIVERSITY OF GLASGOW

LECTURESHIP IN BOTANY

Applications are invited from experimental biologists for a Lectureship in Botany. No field of research interest is excluded but the work of applicants trained in fields other than Botany (e.g. Virology, Molecular Genetics, Environmental Physics, etc.) must be associated with plant tissues after appointment. The appointment will begin as soon as possible and no later than October 1, 1979. The initial salary will be £3,883 to £5,604 (interim) on the Lecturers' scale.

Further information about the post and department may be obtained from Professor M. B. Wilkins.

Applications (8 copies), giving the names and addresses of three referees, should be lodged with the Secretary of the University Court, (Room 18). The University of Glasgow G12 8QQ, not later than December 31, 1978.

In reply please quote Ref. No. 4111/1M. 8302(A)

UNIVERSITY OF BRADFORD

CHAIR

IN THE FIELD OF MEDICAL SCIENCES

Applications are invited for the above post. Further particulars from the Registrar, University of Bradford, Bradford BD7 1DP, West Yorkshire. Closing date for receipt of applications Friday, December 15, 1978. 8333(A)

UNIVERSITY OF BRADFORD

UNIVERSITY OF SURREY

RESEARCH PROJECT ON THERMOCHEMICAL STUDIES ON THE INTERACTION OF SODIUM AND POTASSIUM WITH BIOLOGICAL ACTIVE COMPOUNDS AND BIOLOGICAL TISSUES

Applications are invited for a Research Officer post on the above project, tenable for one year.

Applicants should be under 26 years of age, hold a B.Sc. degree in Chemistry or Biochemistry and have a knowledge of biological systems.

Salary: £3,634 per annum. No superannuation.

Applications in the form of a curriculum vitae, together with the names and addresses of two referees, should be sent to the Staff Officer, University of Surrey, Guildford, Surrey GU2 5XH by December 7, 1978.
8329(A)

THE BRITISH INDUSTRIAL BIOLOGICAL RESEARCH ASSOCIATION

requires an

Editorial Assistant

The post will involve all editorial aspects of producing a bimonthly international journal, *Food and Cosmetics Toxicology*, and a monthly *Information Bulletin*. The work also entails some writing of reviews and abstracts. Candidates should be qualified in chemistry with a bias towards biology or in a biological science, and should have a good command of and interest in the English language.

Salary on a scale ranging from £3,990 to £7,390 per annum. Superannuation scheme in operation.

Applications (handwritten) should be sent to:—

The Assistant Editor,
The British Industrial Biological
Research Association,
Woodmansterne Road,
Carshalton, Surrey SM5 4DS.

8350(A)

IMPORTANT RESEARCH DIVISION OF AN INTERNATIONAL PHARMACEUTICAL GROUP

located in PARIS is offering an

EXCELLENT CAREER OPPORTUNITY for a

TECHNICAL PLANNING OFFICER

to join its BIOLOGY DEPARTMENT

The successful candidate will be responsible for:

a) advising on the implementation of the GLP standards

b) assisting with the planning of research projects

c) organizing the collation and storage of research data

Applicants should have a degree in the Biological Sciences. Past or present experience in the pharmaceutical industry is essential.

An excellent salary and comprehensive benefit program accompany this position.

Please, send Curriculum Vitae to Box No. 8310(A)
NATURE
3 Dyer's Buildings
Holborn EC1N 2NR

8310(A)

BRITISH ANTARCTIC SURVEY

HIGHER SCIENTIFIC OFFICER/ SENIOR SCIENTIFIC OFFICER (BIOMETRICIAN)

An experienced BIOMETRICIAN is required by the Life Sciences Division of the British Antarctic Survey to take part in a number of research programmes, including the offshore biological programme which is a major investigation into physical and chemical features of the marine environmental, phytoplankton standing, crop and photosynthesis and aspects of krill, fish, bird and seal biology at South Georgia.

The management of these and other data bases, development of analytical methodology and provision of general biometrical advice will be amongst the successful applicants duties.

Qualifications: A degree in a mathematical or biological subject; plus at least two years relevant postgraduate experience for appointment as H.S.O., or at least four years for appointment as S.S.O.

Salaries: H.S.O., £4,101 to £5,448 per annum; S.S.O., £5,154 to £6,898 per annum. Starting salary may be above the minimum, according to age and experience.

The staff of the Council are not Civil Servants, but their pay and conditions of service are similar to those of the Civil Service. There is a non-contributory superannuation scheme.

For further details and an application form, please write stating full qualifications and experience to:

The Establishment Officer
British Antarctic Survey
Madingley Road, Cambridge CB3 0ET

Please quote Ref: BAS105.
Closing date: 30 November, 1978.
NATURAL ENVIRONMENT RESEARCH COUNCIL.

8379(A)

LABORATORY TECHNICIAN, OXFORD

Medical lab. tech. graduate preferred, trained in light and transmission electron microscopy required on January 1, 1979 to work in Oxford. Knowledge of day-to-day electron microscope an advantage. Manufacturer's contract for regular major service and breakdown maintained. Initial three-year contract on current Whitley Council scales. Apply for further details to: Director, Lepra, Fairfax House, Causton Road, Colchester, Essex CO1 1PU. 8367(A)

THE ROYAL VETERINARY COLLEGE

University of London
Division of Clinical Studies
DEPARTMENT OF MEDICINE

LECTURER IN VETERINARY MEDICINE

This vacancy is open to veterinary surgeons of some experience. The appointee will work in the department at North Mymms, Hertfordshire, and will be expected to take part in the general clinical work of the department but will have special responsibility in the area of cardiopulmonary disease of the companion animals. The appointee will be required to give instruction to undergraduates and pursue research in this speciality for which the department can provide excellent facilities.

SALARY SCALE (under review): £4,333 to £8,204 including London Allowance. Initial salary will be determined according to qualifications and experience. Superannuation under the Universities' scheme. Removal expenses up to £800 may be reimbursed in approved circumstances.

Further information can be obtained from Professor F. R. Bell, Head of the Department of Medicine—telephone Potters Bar 55486.

Application form and further written particulars available from the Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU (telephone 01-387 2898).

Applications must be received by November 30, 1978. 8292(A)

APPOINTMENTS WANTED

MARINE GEOPHYSICIST GEOLOGIST

20 years experience in off-shore oil, mineral search, teaching and research in Caribbean, Canada and Middle East. Available on short notice. Box No. 8308(A) c/o Nature, 8 Dyers Buildings, Holborn, London EC1N 2NR.

APPOINTMENTS VACANT

LKB
WALLAC

TECHNICAL WRITER FINLAND

WALLAC OY, a member of the international LKB group of companies requires an experienced technical writer for their marketing department.

The successful candidate will have a qualification in electronic engineering and preferably a knowledge of microprocessors and of primary concern an ability to communicate.

The position will be located in Turku which is a pleasant town in south west Finland.

A competitive salary with four weeks paid holiday, free medical care and subsidised meals are amongst the benefits offered. Assistance with relocation expenses will also be considered.

For further information please contact in the first instance: Mr P. L. Morris, LKB Instruments Ltd., 232 Addington Road, Selsdon, South Croydon, Surrey. Tel. No. 01-657 8822. 8368(A)

UNIVERSITY OF LONDON KING'S COLLEGE

TECHNICIAN GRADE 5

required in the Anatomy Department's Electron Microscope Unit. The successful applicant would have overall responsibility for the operation of the E.M. Unit and would also be responsible for the training of junior staff and advising academic staff, undergraduate and postgraduate students on the application of EM techniques. At present the Unit has one AEI EM 6B, and a JEOL 100CX with scanning facility. Applicants should possess an H.N.C. or equivalent qualification and relevant experience. Salary on scale £3,651 per annum rising to £4,185 per annum inclusive (under review). Four weeks annual holiday. College contributory pension scheme. Apply in writing with full details to: The Head Clerk, Ref. 204035/N, King's College London, Strand WC2R 2LS.

8330(A)

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

CHAIR OF PHYSICAL CHEMISTRY

Applications are invited for appointment to the Chair of Physical Chemistry on the staff of the Department of Chemistry.

The salary will be in the range R12,474 to R15,939 per annum, the initial salary to be determined according to the qualifications and experience of the successful applicant. Benefits include an annual bonus, pension and medical aid facilities and a housing subsidy (if eligible).

The University's policy is not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race, religion or colour. Further particulars relating to this practice and to the above post are included in an information sheet obtainable from the London Representative, University of the Witwatersrand, Chichester House, 278 High Holborn, London WC1V 7HE, or from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg 2001, South Africa, with whom applications should be lodged by January 5, 1979. 8327(A)

Drug Metabolism

Section Heads

Biosciences Research Centre, Great Burgh, Epsom

As a result of the Company's expanding research activities, a new Drug Metabolism Unit has been created at the recently established Biosciences Research Centre at Great Burgh, near Epsom. The Unit which forms part of the Clinical Pharmacology Department will be equipped to a high standard and should be ready for occupation early in 1979. Its responsibilities will include the investigation of drug metabolism in experimental animals and man, with particular interest in the areas of biosciences and chemotherapeutic agents.

Three Section Heads are required who will assist the Head of the Unit in the equipping of the laboratories and the appointment of staff within their respective sections before the Unit becomes fully functional.

Analytical Biochemistry -

The Head of Analytical Biochemistry Section will be responsible for a team concerned with the development and application of methods for the quantitative analysis of drug substances and their metabolites in biological systems and for the design and initiation of pharmacokinetic studies. Candidates for this position should have broad experience in the use of H.P.L.C., G.L.C., GC-MS, automated analysis and other relevant analytical techniques used in modern biochemical analysis.

Chemistry-

The Head of Chemistry Section will be responsible for a team involved in the preparation of compounds labelled with radioactive or stable isotopes, which are required for metabolic studies in laboratory animals and man, and for preparing compounds necessary for progressing the Unit's activities. Candidates should have at least three years post-doctoral research experience in synthetic organic chemistry and possess a sound knowledge of the theoretical concepts of the subject. Experience in radio-chemical synthesis and in working with small amounts of substances would be a distinct advantage.

Metabolic Biochemistry -

The Head of Metabolic Biochemistry Section will be responsible for a team investigating the metabolic fate of pharmacological agents in laboratory animals and man. The successful candidate will have had at least three years post-doctoral experience in the field, be well informed on the theoretical concepts involved and possess a substantial knowledge of relevant practical techniques.

The nature of the work in the Drug Metabolism Unit will necessitate considerable liaison between the three Section Heads, the Head of the Unit, and other scientific staff.

The positions which are open to male and female candidates will command a competitive starting salary and a range of other benefits including non-contributory pension and free life assurance.

For an application form please telephone Burgh Heath 53344, Extension 208, or write to: **The Site Personnel Manager, Beecham Pharmaceuticals, Research Division, Biosciences Research Centre, Great Burgh, 1 Yew Tree Bottom Road, Epsom, Surrey.**

Beecham
Pharmaceuticals

THE UNIVERSITY OF SHEFFIELD
DEPARTMENT OF ELECTRONIC AND ELECTRICAL ENGINEERING

SENIOR RESEARCH ASSISTANT

Centralised Facility for Epitaxial growth of III-V Compounds

The Science Research Council has recently established in the Department a centre for the growth of III-V compounds, initially based on GaAs and InP and their alloys, by liquid phase epitaxy. The Senior Research Assistant will be responsible for helping to set up facilities for growing such layers and for investigating problems associated with their growth. There will be close interaction with other members of the existing group working on device and transport properties of these materials. Applicants, men or women, should preferably have a higher degree and significant experience of crystal growth. Tenable four years in the first instance; starting salary in range £3,883 to £5,367 depending on age and experience. Details from Professor P. N. Robson, Department of Electronic and Electrical Engineering (Tel. 0742-78555, Ext. 149) to whom one copy of a curriculum vitae should be sent. Quote ref. R 222/G.

8353(A)

UNIVERSITY OF NAIROBI—KENYA

Applications are invited for the post of

SENIOR LECTURER

in the
DEPARTMENT OF PHYSICS

Applicants should possess a Ph.D. in Physics and have considerable University teaching and research experience as evidenced by publications. Active fields of research in the Department are Theoretical and Solid State Physics, ionospheric physics, palaeomagnetism, and applied geophysics. Any experience in one of these areas would be an advantage.

Salary scale: K£2,988 to K£3,984 p.a. (K£1=£1.33 sterling). The British Government may supplement salary by £4,770 p.a. (sterling) for a married appointee or £3,252 p.a. (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowance and holiday visit passages. S.S.S.F. or F.S.S.U.; family passages; medical aid scheme; housing allowance; various allowances.

Detailed applications (2 copies) including a curriculum vitae and naming 3 referees should be sent by air mail not later than December 12, 1978 to the Registrar (Recruitment and Training), University of Nairobi, P.O. Box 30197, Nairobi, Kenya.

Applicants resident in U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

8383(A)

SALFORD AREA HEALTH AUTHORITY (TEACHING)
HOPE HOSPITAL
(University of Manchester School of Medicine)
Eccles Old Road
SALFORD M6 8HD

RESEARCH TECHNICIAN

required for the recently formed Department of Rheumatology. The job will involve the general running of the laboratory and assistance with research into inflammatory aspects of rheumatoid and other inflammatory conditions. Experience in tissue culture and/or immunological techniques would be preferred.

Salary will depend on qualifications and experience and it is hoped that the successful candidate will be appointed on the salary scale £3,261 rising to £4,680.

Application forms can be obtained from the Sector Administrator, telephone number 061-789 7373, Ext. 202 to whom they should be returned by November 27, 1978.

8328(A)

At Walton Oaks research is directed towards both animal disease and human metabolic disorders. The group concerned with the latter will relocate to our new site at Great Burgh (4 miles from Walton Oaks) in 1979. Within our multi-disciplinary project teams we now require:

PhD Chemists

Within three of our research teams opportunities exist to participate in the planning and execution of the synthetic organic chemistry programmes. Applications are invited from newly qualified PhD Chemists and from those with up to 2 years post-doctoral experience. B.Sc Chemists with a good honours degree (1st or 2(i)) and 3-5 years' relevant post graduate experience are also invited to apply.

B.Sc Biochemists

Two vacancies exist for graduates who within the last two years have achieved a good honours degree in biochemistry or physiology/biochemistry. The first position (for which HNC/D candidates with appropriate experience will also be considered) involves the primary and secondary screening of hypolipidaemic compounds. The person appointed to the second position will assist in the study of platelet function with respect to arterial thrombosis.

Post-Doctoral Biochemist for Diabetes Research

This is a senior position within a group studying maturity on set diabetes and the successful applicant is likely to have undertaken 2-3 years' post doctoral work in diabetes research or a closely related field. Applicants must be capable of initiating a research programme and of motivating the staff involved in its execution.

The men and women recruited to the above positions will enjoy excellent working conditions including flexible working hours.

Please submit full personal and career details (including current salary) to the following, or telephone for an application form:

Mrs M. R. Minchin,
Beecham Pharmaceuticals Research Division,
Walton Oaks, Dorking Road,
TADWORTH, SURREY
Tadworth 4444 (or 2444)

8384(A)

Beecham
Pharmaceuticals

M.R.C. CLINICAL RESEARCH CENTRE
(Northwick Park Hospital)
Watford Road, Harrow
Middlesex HA1 3UJ

The Division of Anaesthesia require a Clinical Scientist to study high pressure neurological syndrome. The appointee will collaborate in high pressure experiments on primates in a new pressure chamber facility.

Knowledge of high pressure engineering an asset with experience in one of the following disciplines: Physiology, Neurophysiology, Pharmacology, Undersea Medicine.

The appointment is for three years only and the salary within the range £3,975 to £6,591 plus L.A. Application form and further details from Mrs J. Tucker-Bull quoting ref. 101/1/4281.

Closing date for applications November 30, 1978.

8313(A)

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oxford polytechnic

Department of Construction

Technician – Geology

(£3,279 to £3,651 inclusive of all supplements)

Technician required in the Geology Section of the Department of Construction, whose duties will include special responsibility for curating the teaching collections.

Further particulars and application forms may be obtained from the Deputy Administrator, Oxford Polytechnic, Oxford OX3 0BP.

8290(A)

NEWCASTLE Area Health Authority (Teaching) Newcastle General Hospital, Senior non-medical Biochemist required for the Neurochemistry Department. The duties include specialised diagnostic procedures in neurology and research in neuromuscular disease. Candidates must possess a good honours degree, and a Ph.D. or suitable research experience—particularly in enzymology—would be an advantage. The appointment may be made at basic grade if the candidate considered most suitable has not yet had sufficient experience to warrant senior grading. Applications in own words, quoting names and addresses of two professional referees, to Western Sector Administrator, Newcastle General Hospital, Newcastle upon Tyne NE4 6BE, by November 24, 1978. Further details may be obtained from Dr R. J. T. Pennington, Department of Neurochemistry at the above address. Tel: (0632) 638811 Extn. 483. 8306(A)

RESEARCH TECHNICIAN

SCHOOL OF CHEMICAL AND PHYSICAL SCIENCES
Kingston Polytechnic

The technican will be required to work in collaboration with Dr A. G. Skinner on certain aspects of the chemistry of flame retardants used in synthetic polymers. The work, which is supported by the S.R.C., will be aimed at understanding the mechanisms of flame retardancy and will include polymer synthesis, testing and thermal decomposition studies. A knowledge of chemistry to degree or equivalent level is preferred.

The appointment is on a temporary basis, up to 2 years only, at T2 grade £3,564 inc.

Application forms, to be returned as soon as possible, from the Assistant Registrar (personnel), Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. 01-549 1366.

8356(A)

THE UNIVERSITY OF SHEFFIELD
RESEARCH FELLOW IN THEORETICAL CHEMISTRY

A vacancy occurs at postdoctoral level in a research group working on theories of the electronic structure and properties of molecules. Tenable immediately for one year with possible renewal. Salary in range £3,883 to £4,631 a year. Applicants, men or women, should have some experience (pre- or post-doctoral) in the field and should write directly to Professor R. McWeeny, Department of Chemistry, The University, Sheffield S3 7HF, with a curriculum vitae and the names of two referees. Quote ref. R 223/G. 8357(A)

PLYMOUTH POLYTECHNIC

School of Environmental Science

SL/LII MICROBIOLOGY

Salary scale:
£4,101 to £7,572

To contribute to the teaching of microbiology in the Biological Sciences degree (with special honours option in Microbiology) to H.N.D. and H.N.C. courses in Applied Biology and the M.Sc. degrees in Applied Fish Biology.

Preference will be given to candidates with experience in Virology.

LECTURER II ECOLOGY

Salary scale:
£4,101 to £6,558

Should be honours graduates in Biology or Environmental Sciences with a strong ecological background and a Ph.D. or equivalent research experience to take part in and organise undergraduate courses in Marine, Freshwater and/or Terrestrial Ecology. There will be opportunities to develop special teaching interests in honours degree options in Biological Sciences and Environmental Science and/or the Masters degree in Applied Fish Biology. Resarch in a suitable topic will be encouraged.

LECTURER II INORGANIC CHEMISTRY

Salary scale:
£4,101 to £6,558

Applicants should be suitably qualified with a broad knowledge of Inorganic Chemistry. Some specialisation in Analytical, Pollution or Marine Chemistry would be an advantage.

Application forms to be returned by **November 24, 1978** can be obtained with further particulars from the **Personnel Officer, Plymouth Polytechnic, Drake Circus, Plymouth PL4 8AA.**

8324(A)

BIRKBECK COLLEGE
(University of London)
DEPARTMENT OF
CRYSTALLOGRAPHY
TECHNICIAN GRADE 2B

uired to assist in the experimental rk of the Liquid Group whose re- rch is directed at the structure of uids, and their role in biological l inorganic environments. Applicants st be qualified (City and Guilds, N.C. or equivalent) and possess a od scientific background. Experience design and construction of apparatus advantage. Opportunities for ther study.

Salary within scale £2,994 to £3.345 der review). 26 days annual leave. rmanent pensionable post.

Apply stating age, qualifications and perience to Administrative Assistant), Birkbeck College, Malet Street, ndon WC1E 7HX or telephone 580 6622, ext. 271 for application rm. 8377(A)

NIVERSITY OF GLASGOW
WELLCOME
SURGICAL INSTITUTE
RESEARCH ASSISTANT

Applications are invited from aduates with Honours in Physiology Pharmacology to work on a 3-year ant-aided project concerning the ect of amines on regional cerebral ood flow and metabolism. The ccessful candidate will be a member a long established and inter- tionally recognised team studying the rebral circulation. Excellent labora- ry facilities and modern equipment. pportunity to study part-time for .D.

Initial salary will be £3,384 on nge 1B of the salary scales for search and Analogous Staff.

Applications to Dr A. M. Harper, ellcome Surgical Institute, University Glasgow, Garscube Estate, Bearsden oad, Glasgow G61 1QH.

In reply please quote Ref. No. 80M. 8378(A)

QUEEN ELIZABETH COLLEGE
Kensington
(University of London)
PHYSIOLOGY DEPARTMENT
TECHNICIAN GRADE 4

A vacancy will exist from January , 1979, for a Grade 4 Technician join a group serving the needs of oth teaching and research within the hysiology Department.

Candidates should possess C. & G. art II, O.N.C. or higher qualifica- ons and experience in either teaching r research laboratory work. Photo- raphic skills would also be an ad- antage.

Salary including London Weighting ithin the range £3,441 to £3,867 p.a. Contact Mrs G. M. Howard for appli- ation form, Queen Elizabeth ollege, Campden Hill Road, London 7AH. Tel: 01-937 5411 ext. 499. 8349(A)

UNIVERSITY OF OXFORD
NUFFIELD DEPARTMENT OF
BSTETRICS and GYNAECOLOGY
UNIVERSITY OF OXFORD
Applications are invited for a
POSTDOCTORAL RESEARCH ASSISTANT

work in the field of reproductive mmunology. Applicants should have perience in cellular immunology. he area being investigated comprises aternal cell-mediated immunity to tal and placental antigens in normal d abnormal human pregnancy.

Salary in range £3,883 to £5,604 epending on age. Grant support is r 3 years.

A curriculum vitae with the name d address of two referees should be nt by December 15, 1978, to Dr . W. G. Redman, John Radcliffe ospital, Headington, Oxford OX3 DU, from whom further information ay be obtained. 8266(A)

UNIVERSITY COLLEGE OF NORTH WALES BANGOR

Applications are invited for the post of
LECTURER IN BOTANY
in the College's School of Plant Biology.

The successful applicant will be expected to teach courses in plant morphology and anatomy and perhaps also ultra-structure, and will have the opportunity to develop new courses in areas of his/her special interests.

The appointment will be to the Universities' Lecturer Scale: £3,883 to £7,754 per annum.

Applications (two copies), giving details of qualifications and experience, together with the names and addresses of three referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, from whom further particulars may be obtained.

Closing date for applications: November 20, 1978. 8297(A)

Radio Astronomer

The National Institute for Telecommunications Research has several vacant positions at the Radio Astronomy Observatory, Hartebeesthoek, Republic of South Africa. The Observatory is active in a wide range of work covering astronomical research, development of electronic instrumentation for radio astronomy and the development of computer software for real time control of observing programmes and for data reduction.

The Observatory is situated 65 km north-west of Johannesburg and has a computer controlled 26-m radio telescope. Cryogenic receivers are available for 21, 18, 13 and 6 cm wavelengths. Additional equipment includes continuum radiometers, a digital correlation spectrometer and a Mark II c VLBI terminal. A mini computer is available for off line programme development and for data analysis.

Some experience in radio astronomy is desirable, and a background in spectral line work or in very long baseline interferometry would be a strong recommendation. However, applicants with a background in electronics and computer science would also be considered. Applicants with adequate experience would be considered for the position of Deputy Head of the Observatory.

For further information and the prescribed application form, please telephone London 01-242 1766. Alternatively, an application form may be obtained from THE OFFICE OF THE SCIENTIFIC COUNSELLOR, CHICHESTER HOUSE, 278 HIGH HOLBORN, LONDON WC1 7HE.
(ref no 78/800/166)

Council for Scientific and Industrial Research (CSIR)

8375(A)

CHARING CROSS HOSPITAL
(Fulham)
BASIC GRADE PHYSICIST

Applications are invited for the above vacancy at this teaching hospital. Applicants should have a first or second class honours degree in physics and previous experience in hospital physics is essential.

The duties will be mainly concerned with the measurement and application of radiations in the medical field. The successful applicant will also be expected to participate in the development of clinical instrumentation, and experience in the use of computers would be an advantage.

Salary within the range—£4,152—£5,088

Further information and an application form available from
District Personnel Department, Charing Cross Hospital, Fulham
Palace Road, W6 9HH. Tel. 01-748 2040 Ext. 2997.

Closing date for receipt of applications 8th December 1978.

8364(A)

UNIVERSITY OF WESTERN AUSTRALIA
Perth
MATHEMATICS
Applications are invited for appointment as
TUTOR AND SENIOR TUTOR
in the Department of Mathematics. These are temporary positions which will be available from January 1, and will extend to December 31, 1979. The appointees will be expected to assume duty not later than February 25, 1979. Further information concerning the Department's staff and research interests may be obtained from the present Chairman, Professor A. L. Blakers.

The current salary ranges are: Senior Tutor—$A13,083 to $A14,987 p.a.; Tutor—$A10,788 to $A12,702 p.a. The appointees will be entitled to an allowance of up to $A1,975 towards appointment expenses.

Applications in duplicate stating full personal particulars, qualifications and experience should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia, 6009, by November 25. Candidates should request three referees to write immediately to the Staffing Officer. 8343(A)

ROYAL POSTGRADUATE MEDICAL SCHOOL
DEPARTMENT OF ANAESTHETICS

Technician with H.N.C. or similar qualification required for this very active research department. The person appointed will be expected to be capable of assuming responsibility for cardio-respiratory and blood gas measurements. Experience in gas chromatography and bio-chemistry is desirable. Starting salary within the range £4,009 to £4,854 depending on experience and qualifications, good holidays and contributory superannuation scheme.

Application forms may be obtained from the Personnel Office, Royal Postgraduate Medical School, 150 Du Cane Road, London W12 0HS quoting reference number 14/104/N. 8342(A)

UNIVERSITY OF LEICESTER
RESEARCH TECHNICIAN (GRADE III)

Applications are invited for the above post to work on the growth energetics of thermophilic and psychrophilic bacteria.

Salary on the scale £2,688 to £3,060.

Applications enclosing a curriculum vitae and the names of two referees, should be made as soon as possible to Dr C. W. Jones, Department of Biochemistry, University of Leicester, Leicester. 8312(A)

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF BOTANY

Applications are invited for a Lectureship in this Department from candidates with interests in Higher Plant Genetics. Within this field preference will be given to candidates with a particular interest in plant reproduction.

The salary will be in the range £3,883 to £7,754, but the initial appointment will be made in the range £3,883 to £4,383 per annum (interim scales).

Further particulars and forms of application, returnable not later than November 30, 1978, from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD. Ref No. 652. 8305(A)

UNIVERSITY OF NAIROBI—KENYA
Applications are invited for the following posts in the
DEPARTMENT OF AGRICULTURAL ECONOMICS
PROFESSOR

Applicants must possess a good honours degree in Agricultural Economics, Economics or Agriculture and a Ph.D. in Agricultural Economics. Relevant teaching and research experience are essential. Academic duties will mainly concern lecturing and research supervision of M.Sc. and Ph.D. students. The appointee will be responsible for further development of the undergraduate curriculum and organisation of M.Sc. research.

ASSOCIATE PROFESSOR

Applicants must possess a Ph.D. in Agricultural Economics or Economics. Candidates should have considerable experience in teaching and research as substantiated by published work. Specialisation in Agricultural marketing and/or marketing of Food products will be added advantages. Academic duties will mainly concern lecturing and research supervision of M.Sc. and Ph.D. level.

Salary scales: Professor K£4,632 to K£5,562 p.a., Associate Professor K£3,864 to K£4,488 p.a. (K£1=£1.33 sterling.) Family passages; S.S.S.F. or F.S.S.U.; medical aid scheme; subsidised housing/housing allowance; various allowances.

Detailed applications (2 copies) including curriculum vitae and naming 3 referees should be sent airmail to Registrar (Recruitment and Training), University of Nairobi, P.O. Box 30197, Nairobi, Kenya. Closing date: December 12, 1978.

Applicants resident in U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 8381(A)

UNIVERSITY OF SOUTH FLORIDA

CHAIRMAN OF THE MARINE SCIENCE DEPARTMENT

The Department of Marine Science of the University of South Florida located at St Petersburg announces the availability of the position of Chairman of the Department. Marine Science at USF has been designated by the Board of Regents of the State University System for special funding by the State Legislature for its development as a graduate Center of Excellence.

The Department now consists of ten faculty members. Six additional faculty members are being recruited. The Department's primary mission is research and graduate training.

The Chairman should be a Ph.D. with excellent research credentials and considerable administrative experience. A background of work in a marine station or oceanographic institute is highly desirable. The rank is Professor. Salary will depend on experience and professional accomplishment but the 12 month minimum is $35,000. The appointment can be made at any time during the academic year. The closing date for the receipt of applications is January 1, 1979.

Applicants should send complete résumés to Dr L. T. Hopkins, Chairman of Search Committee, Department of Marine Science, University of South Florida, St Petersburg, Florida 33701. The University is an affirmative action equal opportunity employer.

8242(A)

STATE OF KUWAIT

Kuwait University

Advertisement for Chief Technician Post—Geology Department

A highly qualified technician is needed for the departmental laboratory workshop. The position involves routine processing of standard geological services (thin sections, core treatment, grain mounting, etc . . .) and organisation and supervision of the workshop. The successful candidate must possess basic knowledge in repair and maintenance of electrical laboratory appliances and instruments. A B.Sc. in geological or physical sciences is desirable. Strong command of the English language is essential. Send résumé to: **Chairman, Geology Department, Kuwait University, P.O. Box 5969, Kuwait.** 8335(A)

LINCOLN COLLEGE

(University College of Agriculture)

NEW ZEALAND

CHAIR IN HORTICULTURE

The Council of Lincoln College, University College of Agriculture, invites applications for the position of Professor of Horticulture and Head of the Department of Horticulture, Landscape and Parks. This Chair has become vacant on the appointment of Professor T. M. Morrison to the position of Director of the Queensland Agricultural College.

Applicants should hold an advanced degree in a field appropriate to any of the responsibilities of the Department which cover horticultural sciences, production, management, parks and recreation, and landscape architecture, and should have an established record in teaching and/or research.

The successful applicant will be appointed as Head of Department and will be responsible to the College Principal for the administration of his Department. Previous administrative experience will therefore be taken into consideration.

The appointment will be made within the existing range of professorial salaries, NZ$23,012 to NZ$29,188 per annum, the commencing salary being determined by the College Council. Academic salary scales are at present under review.

Expenses of appointment reimbursed up to specified limits. New Zealand Government Superannuation is available.

Further details and General Conditions of Appointment are obtainable from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the College.

Applications close on January 15, 1979. Please quote Vacancy No. 78/60. 8294(A)

CELL SURFACES

Applications are invited for a three-year postdoctoral position available from January 1, 1979 for research on animal lectins and cellular interactions. Salary £4,130 to £4,601.

Applications with names of two referees to Dr G. M. W. Cook, Department of Pharmacology, Hills Road, Cambridge CB2 2QD. 8365(A)

UNIVERSITY OF OXFORD

DEPARTMENT OF ZOOLOGY

Appointment of a

DEPARTMENTAL DEMONSTRATOR IN DEVELOPMENTAL BIOLOGY

Applications are invited for the post of Departmental Demonstrator in Developmental Biology. The person appointed would be expected to take up the post by October 1979. The successful candidate, who should recently have received a Degree of Doctor of Philosophy or be in his/her final year before submission, will be required to take part in developmental biology teaching and to undertake research in developmental biology.

The appointment will be for three years in the first instance with the possibility of renewal for a maximum of a further three years. The salary subject to review will be within University scale £3,384 per annum to £4,466 per annum with membership of U.S.S.

Applications, including the names of two referees, should be submitted to Head of Department, Department of Zoology, South Parks Road, Oxford OX1 3PS, by December 15.

8338(A)

FELLOWSHIPS

UNIVERSITY OF NEWCASTLE UPON TYNE FELLOWSHIPS

Applications are invited for the following Fellowships (tenable for two years from October 1, 1979) from persons who have shown themselves able to carry out original research. The value of each Felllowship is £3,883 in the first year and £4,133 in the second year (under review).

SIR JAMES KNOTT FELLOWSHIPS

TWO Fellowships available in any faculty.

WILFRED HALL FELLOWSHIP

ONE Fellowship available for research in the Faculty of Science.

EARL GREY MEMORIAL FELLOWSHIP

ONE Fellowship available for research in any faculty. As a general rule this Fellowship will be awarded to candidates who have graduated from the University of Newcastle upon Tyne.

Further particulars and application forms (which must be returned by December 31, 1978) may be obtained from the Registrar, University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne NE1 7RU. 8345(E)

UNIVERSITY OF WARWICK POSTDOCTORAL RESEARCH FELLOW

in Molecular Beam Scattering

Applications are invited for an SRC Postdoctoral Research Fellowship in the Department of Chemistry and Molecular Sciences to work on the investigation of simple ion molecule reactions using a crossed molecular beam apparatus in collaboration with Dr D. M. Hirst. Applicants should have experience in electronics, high vacuum work and preferably in molecular beams. The post, which is available immediately, will be for one year in the first instance, with the possibility of renewal for a further year. Salary on the Range 1A scale: £3,883 to £6,555 p.a. Application forms and further particulars from the Academic Registrar, University of Warwick, Coventry, CV4 7AL to whom applications should be returned as soon as possible. Please quote Ref. No: 14/3A/78. 8383(E)

EMBO

European

Molecular Biology Association

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY SPRING 1979 AWARDS

Next deadline: February 20, 1979

The long term fellowships will usually be for a period of up to one year but applications for a renewal for a second year are considered. To be eligible a candidate must hold a doctors degree. Preference will be given to European and Israeli applicants wishing to work within Europe or Israel. EMBO long term fellowships are NOT, however, awarded for exchanges between laboratories within any one country. Applications for fellowships to be held outside Europe and Israel are considered but they have a lower priority, as do applications from non-European scientists wishing to work in Europe or Israel.

The deadline for applications for the 60 fellowships to be awarded in April 1979 is February 20, 1979. Because the selection procedure involves an interview this deadline should be respected.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organisation, 69 Heidelberg 1, Postfach 1022.40, F.R. Germany. 8317(E)

UNIVERSITY OF ILLINOI

AT URBANA

Urbana, Illinois

HIGH-FIELD N.M.R. POSTDOCTORAL FELLOWSHIPS

Applications are invited fc postdoctoral work on the NMR c systems of chemical and biologic interest, under the direction c Professor Eric Oldfield. Fourie transform spectrometers equippe with widebore super-conductin magnets, operating at 360, 220 an 150 MHz, are available for use o the following projects:

1) Quadrupole-echo studies c specifically ^{2}H-labelled mebrane Topics of interest include the stud of protein-lipid interaction in th cytochrome oxidase system and re laxation studies in liquid crystals.

2) Phosphorus-31 NMR studie of protein-lipid interaction in mode and biological membrane systems

3) High-resolution ^{13}C NMI spectra of protein crystals usin high-power decoupling and magic angle sample-spinning techniques together with studies of protein structure in solution using large sample sideways-spinning tub probes.

4) Development of a LN$_2$ an then a LHe cooled-coil probe fo room temperature high resolutio NMR.

Applications, consisting of curriculum vitae, list of publica tions, and the names and addresse of three referees should be sent to

Professor Eric Oldfield
School of Chemical Sciences
University of Illinois at Urban
Urbana, IL 61801, U.S.A.

The University of Illinois is a Equal Opportunity / Affirmativ Action employer. 8291(E)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

GEOPHYSICS AND PLANETARY PHYSICS

Applications are invited for a POS DOCTORAL FELLOWSHIP RESEARCH FELLOWSHIP in sm friendly research group with w interests in theoretical global g dynamics and planetary physics: g physical fluid dynamics, planet magnetism, rotation, deformati Appointment will be for one year the first instance, renewable for second year. Send curriculum vi and names of three referees to P fessor M. G. Rochester, Physics D partment, Memorial University Newfoundland, St John's, Newfou land, Canada A1B 3X7.

8362(E)

UNIVERSITY OF SUSSEX

School of Biological Science

RESEARCH FELLOWSHIP IN BIOCHEMISTRY

Applications are invited for a postdoctoral Research Fellowship in Biochemistry to work on an M.R.C. financed project to investigate the mechanism and regulation of RNA synthesis during oogenesis in the amphibian Xenopus Laevis.

The appointment will be fo approximately two years commencing as soon as possible, at an initial salary in the range £3,88 to £6,555 per annum on the Research and Analogous Facult 1A scale, with superannuation benefits.

Applications, giving qualifications, experience and names o two referees, should be sent t Dr T. Beebee, School of Biological Sciences, University of Sussex Falmer, Brighton BN1 9QG.

8339(E)

FELLOWSHIPS

The Wellcome Trust

Wellcome Senior Research Fellowships in Clinical Science

The purpose of these Fellowships is to ensure that workers of high promise as clinical investigators are not deprived of opportunity by lack of an academic vacancy at the appropriate time. This fellowship scheme includes all medically qualified graduates from British universities in clinical and para-clinical departments working on a subject directly related to a clinical problem. Age limit 35 years. Salary according to age and experience within the range of £6,412 — £7,412 per annum (under review).

Wellcome Senior Research Fellowships in Basic Biomedical Science

The purpose of these Fellowships is to provide opportunities for research for medically or scientifically qualified scientists from British universities who have shown special promise in their initial studies of basic biomedical problems. Candidates must not be more than 32 years of age. Salary according to age and experience on a non-clinical scale £4,882 — £6,555.

Applications for both fellowship schemes, which are tenable for up to five years, must be held in an appropriate academic department in the UK, and have to be submitted by the Head of Department in which the candidate proposes to work. **Further details and application forms may be obtained from the Grants Section, The Wellcome Trust, 1 Park Square West, London NW1 4LJ. Tel: 01-486 4902. Completed application forms must be returned by 31st January 1979.** 8299(E)

STUDENTSHIPS

niversity of wales

niversity
ollege of
wansea

Postgraduate Studentships

Applications are invited for two ,R.C. **C.A.S.E. Studentships** in the **epartment of Physics**, one for esearch on the physics of the high oltage spark in conjunction with .E.G.B. Leatherhead, and the ther for an investigation of the hysical processes in pulsed neutron ounters in conjunction with .K.A.E.A. Winfrith.

Applicants should possess a good achelor's or Master's degree from British University in Physics, lectrical Engineering or related ubject, and will be encouraged to egister for a Ph.D. degree of the niversity of Wales.

Further details from Professor . Dutton. Telephone: (0792) 5678, Extn. 390. 8325(F)

THE UNIVERSITY OF SHEFFIELD
DEPARTMENT OF METALLURGY
S.R.C./C.A.S.E. RESEARCH STUDENTSHIPS

Applications are invited from men and women for two Research Studentships.

(i) to study the influence of preferred orientation (texture) on the fatigue behaviour of steels used in heavy automotive applications (in collaboration with the GKN Group Technological Centre).

(ii) to examine void assessment in materials with changing structures during deformation in service (in collaboration with the Central Electricity Generating Board).

Value will be equal to that of a normal S.R.C. award increased by up to £500 per annum depending upon the experience of the successful applicants. It is expected that the holders of the studentships will register for a Ph.D. degree. Applications to Professor G. J. Davies, Department of Metallurgy, The University, Mappin Street, Sheffield S1 3JD by December 8, 1978. Quote ref: R 221/AI.

8341(F)

SYMPOSIA

INTERNATIONAL PEPTIDE SYMPOSIUM

The Intramural Program of the National Institute of Neurological and Communicative Disorders and Stroke will sponsor an International Symposium entitled "The Role of Peptides in Neuronal Function" to be held May 7 to 9, 1979 at the National Institutes of Health reservation in Bethesda, Maryland. The three-day meeting will draw together investigators from diverse disciplines for a comprehensive review of peptide research as it relates to the field of neurobiology.

The first day will include presentations on the anatomy and physiology of peptidergic neurons, as well as the synthesis and secretion of peptides. The second day will begin with a discussion of peptide-mediated communication in the nervous system, followed by reviews of the neurobiology of specific peptides, including angiotensin, neurotensin, somatostatin, substance P, vasoactive intestinal peptide, luteinizing hormone releasing hormone, thyrotropin releasing hormone, olfactory system peptide, bag cell hormone and neurodepressing hormone. The final day will be devoted entirely to the pharmacology, physiology and potential clinical application of opioid peptides.

The Symposium is being organised by Drs Jeffery Barker and Thomas Smith of the N.I.N.C.D.S. Laboratory of Neurophysiology. Those interested in attending should contact either Dr Barker and Dr Smith, Bldg. 36, Room 2C02, National Institutes of Health, 9000 Rockville Pike, Bethesda, Md. 20014. Tel: 301-496-2414.

8307(M)

ASSOCIATESHIPS

HERIOT-WATT UNIVERSITY
DEPARTMENT OF PHYSICS
POSTDOCTORAL RESEARCH ASSOCIATESHIP
S.R.C. C.A.S.E. STUDENTSHIP

Applications are invited from suitably qualified physicists or chemists to join an established group to work in the area of high power laser induced energy transfer processes in molecules, particularly relevant to laser induced isotope separation, photochemistry, and optically pumped molecular lasers.

The postdoctoral appointee should possess a Ph.D. and have experience in infra-red laser physics or molecular spectroscopy.

The salary would be up to £4,382 per annum (under review) and the appointment would be for two years in the first instance.

The C.A.S.E. studentship will be for a period of three years, and the appointee will research for a Ph.D. and should possess a degree in either physics or chemistry.

Applicants should contact Dr C. Pidgeon, Physics Department, Heriot-Watt University, Riccarton, Edinburgh (Tel: 031-449 5111, Ext: 2333) as soon as possible to obtain further information. 8340(O)

APPOINTMENTS VACANT

POSTDOCTORAL POSITIONS

Two positions available immediately d for next summer for Biochemical d Biophysical studies on the regulan and mechanisms of gene expression d the nucleic acid-protein interion. Experience in molecular logy or physical biochemistry irable.

Interested applicants should send rriculum vitae and three letters of ommendation to Dr C.-W. Wu, partment of Biochemistry, Albert stein College of Medicine, Building **205, Bronx, N.Y. 10461. An equal portunity employer.** 8336(A)

UNIVERSITY COLLEGE DUBLIN

Applications are invited by the Governing Body of the College for the following full-time statutory post:

PROFESSORSHIP OF EXPERIMENTAL PHYSICS

Prior to applications, further information (including details of application procedure) should be obtained from Mr J. P. MacHale, Secretary and Bursar, University College, Dublin 4. Tel. 693244, ext. 431.

THE LATEST DATE FOR RECEIPT OF COMPLETED APPLICATIONS IS THURSDAY DECEMBER 7, 1978. 8199(A)

POSTDOCTORAL RESEARCH ASSOCIATESHIPS AT THE NAVAL MEDICAL RESEARCH INSTITUTE BETHESDA, MARYLAND

Sponsored by
The Naval Medical Research and Development Command
in association with
The National Research Council

Competitive applications are now being accepted for postdoctoral research associateships, starting October 1, 1979, in the areas of behavioural sciences, environmental stress, immunology, microbiology, parasitology, pathology, toxicology, and underwater bimedical research. Candidates must have received their doctoral degree (M.D., Ph.D., D.V.M. or Sc.D.) within the last five years. Salary will be $19,263 per annum.

Completed application forms are due not later than January 15, 1979. Information and application forms may be obtained by writing to the National Research Council Associateship Office (JH608-N3), 2102 Constitution Avenue, N.W., Washington, D.C. 20418. An Equal Opportunity Employer.

8318(O)

ASSISTANTSHIPS

UNIVERSITY COLLEGE, GALWAY, IRELAND
RESEARCH ASSISTANTSHIPS (2)

University College, Galway, announces two postgraduate student research assistantships tenable in the Department of Physics and/or the Department of Oceanography for participation in a field and laboratory study of oceanic whitecaps, sea spray, and their influence on the marine atmosphere. Preference will be given to students wishing to pursue dissertation or thesis research on these topics.

These assistantships are available for the present academic year. Salary: £1,500 per annum.

Letters of application, with academic transcripts and the names of two references, should be in the hands of Dr E. Monahan, Department of Oceanography, U.C.G., within two weeks of the date of this advertisement. Successful applicants will be expected to take up their assistantships *not later than 1 January, 1978.* 8374(P)

DEPARTMENT OF PHYSIOLOGY

UNIVERSITY OF BRISTOL
RESEARCH ASSISTANT

Applications are invited for a Postdoctoral Research Assistantship within the Department of Physiology, University of Bristol, for a period of one year in the first inctance, renewable-up to three years. Salary on the scale £3,883 to £4,882 per annum.

The post may be taken up in one of a number of laboratories where the research programmes are concerned with the following topics, muscle spindles, trigeminal pain, developmental neurobiology, simple nervous systems, or flagellar mobility. Many of the research problems involve the use of structural methods as well as or instead of electrophysiological methods. Enquiries may be made to Professor T. J. Biscoe, at the Department of Physiology, University of Bristol, Medical School, University Walk, Bristol BS8 1TD.

Detailed applications, including the names and addresses of three referees should be sent by November 22, to the Registrar and Secretary, University of Bristol, Senate House, Bristol BS8 1TH. 8346(P)

UNIVERSITY OF BRISTOL

DEPARTMENT OF PHARMACOLOGY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a postdoctoral research assistantship funded by The Wellcome Trust for three years from the date of appointment. The project is an investigation of the neuronal basis of stimulus evoked analgesia using electrophysiological techniques. Previous experience of electrophysiological recording from the C.N.S. or peripheral sensory electrophysiology is desirable.

Salary will be on the appropriate academic scales, determined by age and experience (minimum £4,382 p.a. at age 26—scale under review). Applications giving details of age, qualifications and experience, together with the names of two referees, should be sent by November 17 to Dr R. G. Hill, The Medical School, University Walk, Bristol BS8 1TD.

8347(P)

STUDENTSHIPS

UNIVERSITY OF LEICESTER

DEPARTMENT OF PHYSICS

THE PHYSICS OF DISORDERED MATTER
S.R.C. C.A.S.E. STUDENTSHIP

Applications are invited for a C.A.S.E. research studentship with the Department of Physics leading to the degree of Ph.D. The project is a study of liquid semiconductors and alloys using photoelectron spectroscopy and will be undertaken in collaboration with A.E.R.E. Harwell. It provides an opportunity to join an active research group working in a research area of particular relevance to modern technology in which much fundamental physics remains to be discovered.

Applications, which should give the names of two persons to whom reference may be made, are sent to Dr C. Norris, Department of Physics, The University, Leicester LE1 7RH.

8321(F)

COURSES

EMBO LABORATORY COURSE
on
"PLASMIDS—A COURSE IN ADVANCED TECHNIQUES"

APRIL 9 to 29, 1979
University of Erlangen, Germany
Organisers: Kenneth Timmis and Alf Pühler

The course will consist of laboratory experiments and lectures designed to instruct participants in the use of recently developed methods that are particularly important for the genetic, structural and functional analysis of plasmid genomes. These will include transposition mutagenesis, gene cloning, the uses of restriction enzymes, electron microscopy and DNA sequencing for structural analysis, and the uses of minicell and *in vitro* systems for studying plasmid expression and replication.

A mini-symposium on the theme "Plasmids of Medical, Environmental and Commercial Importance" will follow the laboratory course.

Applications, with a short curriculum vitae including future scientific interests, and any information which might help in their evaluation should be sent in duplicate to :

Professor A. Pühler
Institute of Microbiology and Biochemistry
University of Erlangen
Friedrichstr. 33
D 8520 Erlangen, FRG

8376(D)

European Molecular Biology Association
EMBO COURSE
ION-SENSITIVE INTRACELLULAR MICROELECTRODES

Bristol, England,
June 25 to July 8, 1979

The course is for 12 active researchers who wish to learn how to use ion-sensitive intracellular microelectrodes. Participants will be taught how to make, calibrate and do experiments with K^+, Cl^-, Na^+ and pH^+-sensitive microelectrodes. Most of their time will be spent in the laboratory, although there will be some lectures during the course and a two-day round-table discussion at the end. During the round-table, a number of leading workers in the field will describe their methods and recent results. Instructors will include Roger Thomas, Dave Ellis, Richard Vaughan-Jones, Jochen Deitmer, Bill Moody and Andrew Sharp.

Applicants should have some experience of electrophysiological techniques, and have a convincing reason for wishing to take the course and a letter of recommendation.

Acceptance for course will be notified to applicants early in March. Participants will have to pay their own accommodation and subsistence expenses (estimated at about £100), their travel expenses and a Course Registration Fee of £30. One or two grants are available for applicants who can pay only their travel expenses. Accommodation will be arranged by the course organiser.

Applications to Dr R. C. Thomas, Department of Physiology, University of Bristol, Bristol BS8 1TD, England, must be received by February 19, 1979.

8322(D)

APPOINTMENTS VACANT

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM
SCIENTIFIC OFFICER

A Scientific Officer is required at the Plymouth Laboratory to help with current research on cephalopods and marine worms. The successful applicant will have a good degree in Zoology and a keen interest in systematics, morphology and marine ecology. Duties will include many routine tasks and some heavy manual work, particularly during cruises. Salary is on the scale £2,839 to £4,415 per annum. There is a non-contributory Pension Scheme. Applications with full curriculum vitae and giving the names of at least two referees, should be sent to The Director, Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth PL1 2PB, to arrive not later than December 1, 1978.

8198(A)

SIXTH SUMMER PROGRA
IN METHODS OF
IMMUNOLOGIC RESEAR
AND DIAGNOSIS

The sixth biennial program
current methods of immunologic
search and diagnosis will be off
by The Center for Immunology of
State University of New York
Buffalo June 4 to 22, 1979. It
consist primarily of a combination
core and elective practical laborat
exercises to allow the participant
become acquainted with the technic
by personally carrying them out
the bench complemented by demons
tions, lectures and discussions.
entire program is designed to pro
the participant with a survey
presently-available methodology
insight into the underlying immu
logic principles.

Further information may be
tained from James F. Mohn, M.
Director, The Center for Immunolo
State University of New York
Buffalo, 210 Sherman Hall, Buff
New York 14214. The deadline
applications is March 31, 1979.
8319(D

ASSOCIATESHIP

IMPERIAL CANCER RESEARCH FUND DNA TUMOR VIRUS MEETING 1979
CHURCHILL COLLEGE CAMBRIDGE, ENGLAN

July 16-20, 1979

The Meeting will be on th
molecular biology of small DN
tumour viruses, particularly pol
oma, SV40 and adenoviruses an
is open to anyone interested in th
subject. The scientific programm
will include oral presentations an
posters.

The cost for registration, accom
modation, food and the abstrac
book will be approximately £10
Travel money can only be pro
vided in a few, very exceptiona
circumstances.

Anyone interested in attendin
the meeting should apply immedi
ately for further details to Th
ICRF DNA Tumour Virus Meetin
Secretary, Imperial Cancer Re
search Fund, Lincoln's Inn Field
London WC2A 3PX, England.
8360(D)

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JULY 16-20, I.C.R.F., D.N.A. Tun
Virus Meeting, Cambridge (I.C.R.
D.N.A. Tumor Virus Meeting Sec
tary, Imperial Cancer Research Fu
Lincoln's Inn Fields, London WC
3PX, England). 8361(B

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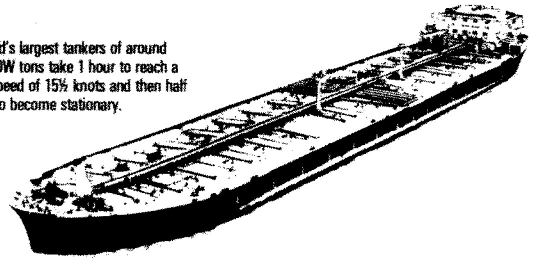

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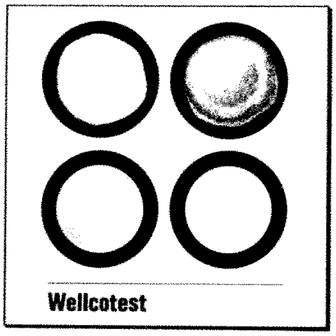

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1 Brit. med. J. (1977) Volume 1, 22nd January.

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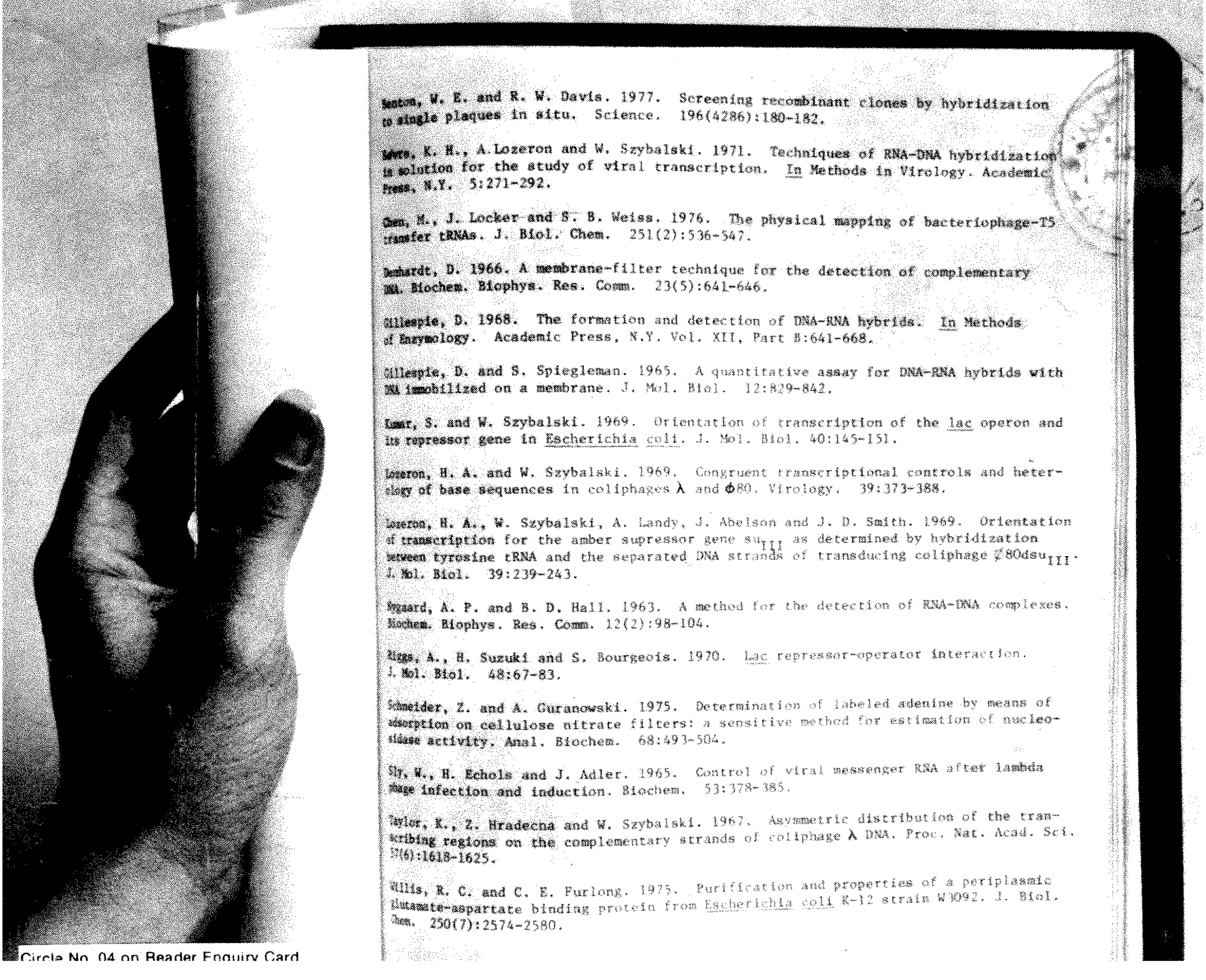

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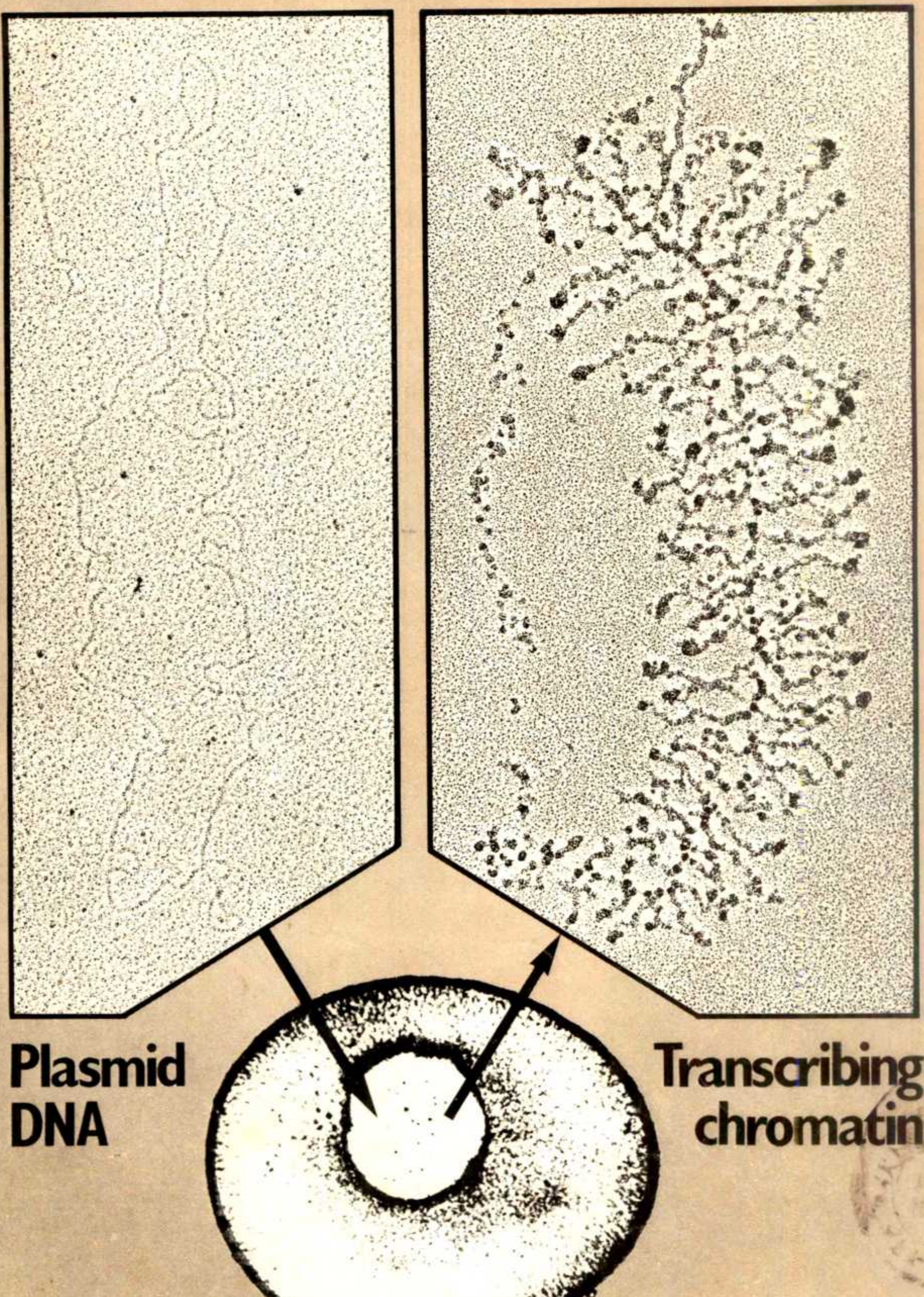

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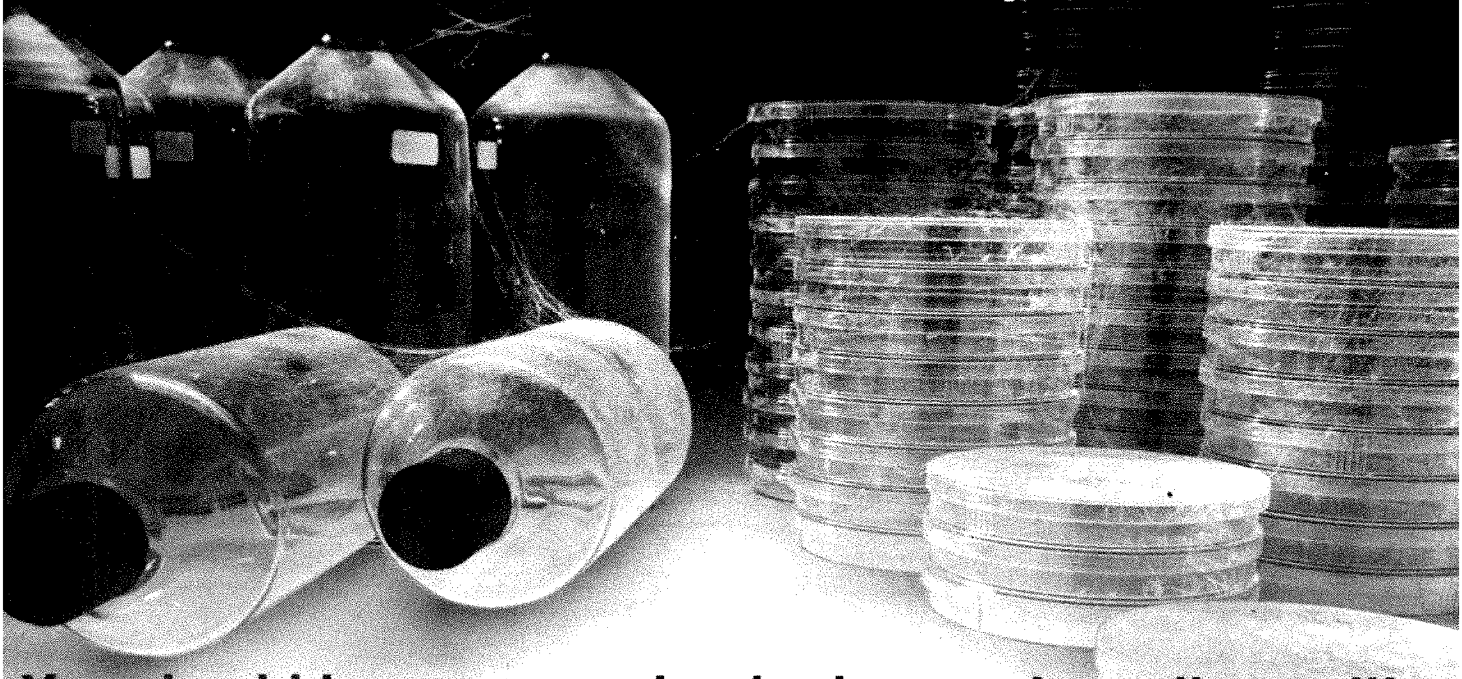

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Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the Système International. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; exp (a) is preferred to e^a if 'a' is more than one character.

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Genetic manipulation: proposed new guidelines for UK

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Nobel Prizes should give encouragement

WITH this year's Nobel Prizes awarded, it may be appropriate now to ask whether there is new ground to be broken in the future by changes in criteria applied to selection for the prizes reflecting changes in the nature of scientific enquiry itself and its relationship to society. Certainly in recent years there has been a cautious and belated recognition of astronomy as a subject appropriate for the physics prize. But there still remain large areas of scientific endeavour yet to be included within the orbit of the prizes, of which the earth sciences is perhaps the most conspicuous example. Within the present terms of award of the prizes these areas are unlikely ever to be recognised by the prize committees.

Many would argue that the prizes ought in any case to be phased out as they represent an outdated reward system, generate a fair amount of bitterness and jealousy, and often convey a spurious air of authority by their award that is misinterpreted not just by the general public but even by the scientists themselves, who should know better. If the prizes are simply seen as the final often belated stamp of approval on a fine piece of work, then these arguments have considerable force. But they surely could be, and (with the exception of the peace prize) rarely are, a way of saying "this activity looks interesting. By awarding it a prize we will be giving it encouragement and will be drawing the attention of a much wider public to it."

It goes without saying that such an approach is much more risky. The work in question may end up in disappointment, and the publicity afforded to it may have been damaging. And much excellent work which at present leads ultimately to the award of a prize will fail to be recognised at the time. But it may well be that an aura of infallibility around the Nobel Prizes could be dispensed with.

As it happens, 1979 would be a particularly appropriate year for the prize committee to explore a new approach. Whatever the expected shortcomings of the United Nations Conference on Science and Technology for Development in Vienna next August, none but the most blinkered will by then be ignorant of the questions being raised about science in and for the developing world. One of the more frequently heard complaints by those involved in scientific work in the developing world is that the seductive attractions of a western-style academic science are too great for too many of the most highly intelligent, who abandon to less competent people the much more immediately relevant problems lacking reward-studded lustre. Nobel Prizes have been one of the ways of conferring this lustre. What could be more appropriate than that in 1979 a serious effort was made to identify work being done in the Third World of wide impact, even if not of exalted scholarship, and give to it an imaginative dose of encouragement. ☐

Victims of censorship

EVERY week roughly 20,000 copies of *Nature* go into the mail. Of these, rather more than 80% leave the United Kingdom for all parts of the globe from China to Peru. As far as we can tell they all reach their destination (barring the odd few that go astray), with one major exception. Certain countries in Eastern Europe take it upon themselves to censor the incoming mail of their citizens.

The pattern is not uniform; some countries don't bother, others are ruthless. By far the biggest offender is the Soviet Union. When there is any mention of that country in our news pages, whether it is favourable, neutral or critical, copies of that particular issue are often simply thrown away at the border. As a result our Soviet readers see, at best, half of all issues of the journal.

There are ways to try to beat the system, of course, but they are only marginally effective and temporary expedients. What is needed, and what is most unlikely to occur, is for governments given to these extraordinary petty gestures to recognise first that all intelligent scientists in their country know perfectly well much of what they are trying to suppress, and second that one of the most effective ways of crippling the growth of science is to inhibit the free flow of scientific literature. ☐

Funding delays handicap ISABELLE in race for new particle

The US Department of Energy has extended construction time for Brookhaven's 400 × 400 GeV accelerator from five to seven years. **David Dickson** reports on the implications.

PHYSICISTS at the Brookhaven National Laboratory on New York's Long Island are trying to persuade the Department of Energy to reverse its decision to extend the construction period of its new 400 x 400 GeV intersecting storage accelerator (ISABELLE).

At stake is a race with the European Organisation for Nuclear Research (CERN) in Geneva to demonstrate the existence of the theoretically predicted intermediate vector boson, known as the W particle. According to the Weinberg-Salam unification theory, this particle carries the weak force in the same way that the photon carries the electromagnetic force.

Initially, Brookhaven's scientists hoped ISABELLE's larger number of interactions would compensate for CERN's planned earlier start in the search for the particle using proton-antiproton interactions. The extra two years' delay in construction could, it is feared, considerably reduce chances of being first past the post.

Construction work on what has been described as the largest basic research project ever built began last month. When completed, the 2½-mile circumference accelerator will provide a range of detection facilities for studying the results of collisions between two beams of opposite circulating protons.

The Department of Energy, under pressure from members of Congress, agreed last year to put $23 million to begin construction work on ISABELLE into its budget request for 1979; when this money officially became available last month, a ground-breaking ceremony took place.

However, in an attempt to keep the lid on funding for high energy physics over the next few years, the department later announced that the accelerator's construction schedule would be increased from five years to seven. The first experiments have been pushed back from 1984 to 1986.

ISABELLE has been designed to make use of pulses of 30 GeV protons from the Alternating Gradient Synchroton, Brookhaven's last major high energy physics facility which came into operation in 1961. The pulses will be stacked sequentially in each ring until 10 amps of circulating current has been built up, at which time the two beams can be slowly accelerated to full energy and made to interact at six separate experimental stations.

The proton collisions will provide a centre-of-mass energy of 800 GeV, equivalent to that available from protons of 340,000 GeV striking protons at rest. ISABELLE will therefore be able to extend by a factor of 10 the centre-of-mass energy range presently available on CERN's Intersecting Storage Ring; it will also have a far greater luminosity (the factor which determines the number of interactions).

ISABELLE has been on the drawing board since 1971, when preliminary designs were first made, and has enjoyed the consistent backing of the US high energy physics community. Official procrastination was broken last year by Representative Jerome Ambro, a Long Island congressman with one eye on science and the other on the jobs and money ISABELLE will bring.

After some political horse-trading that also involved the decision to site the Solar Energy Research Institute in Denver, Colorado, Mr Ambro persuaded Congress to add $5 million to the Department of Energy's authorisation for 1978 to go ahead with ISABELLE—and to defeat attempts by the Office of Management and Budget to have the money deferred.

Further problems were raised when Dr Robert R. Wilson, director of Fermilab, tried to outbid Brookhaven for a major share of the 1979 budget in order to speed up the construction of Fermilab's Energy Doubler. However Wilson's hand was weakened when the department's High Energy Physics Advisory Panel reconfirmed top priority for ISABELLE.

The final hurdle came late in the spring, when the department announced that it had decided to maintain funding for high energy physics at a constant level over the next few years—and that ISABELLE's construction period was to be extended to avoid initial "peaking" that would upset this scheme.

In political terms, the department hopes this will make the high energy physics programme more acceptable to a cost-conscious Congress. However, economically, the result has been to increase the projected overall cost of ISABELLE from $240 million to $275 million; and scientifically it means the extra two-year delay until the first results are produced.

"We would not have built this machine just to discover the W particle," Dr Jim Sandford, project manager for ISABELLE said last week. He points out, for example, that the increased luminosity over CERN's SPS ring, means that Brookhaven will be in a much better position for a detailed analysis of higher energy states, decay modes and so on.

"But I do not want to miss out, if at all possible, in finding it. With our greater luminosity, we had a good chance of finding the particle ahead of CERN on the five-year schedule. On a seven-year schedule, we have considerably less chance."

Brookhaven scientists still hope that, once construction of the accelerator's superconducting magnets and other components is well under way, the Department of Energy can be persuaded to alter its policy and make more money available earlier—saving on costs by reducing labour and avoiding some of the effects of inflation.

So far, the department has remained firm. It has proposed to make available $45 million for ISABELLE's construction costs in the fiscal year 1980, making the total for the first two years still considerably less than the $80 million which the laboratory had proposed for the first year of construction alone. □

Isabelle gets under way: Dale Myers, Assistant Secretary at the Department of Energy, in action at the ground-breaking ceremony

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ESF warns of dangers of shortfall in posts for young scientists

"THERE is a potentially dangerous situation in Europe which is causing young scientists who might otherwise choose a career in research not to do so," Sir Brian Flowers, president of the European Science Foundation (ESF), said last week. He was speaking after the ESF assembly had approved the annual report for 1978.

A recent ESF study has shown that in most of its members' countries, "the majority of those now in employment will stay in office until the beginning of the 1990s when they will reach their normal retirement age. The demand for replacements during the next 15 years will be exceedingly low." The annual report says available posts are now so few that the chance of a junior research assistant getting a permanent university appointment has gone down from 70% in the 1960s to 15% this decade.

The problem of making posts available to young research workers is well-known. The ESF draft report adds one extra argument. In the past, it says, 65% of a university's budget was spent on personnel and the remaining 35% on maintenance, administration, upkeep of the library and research. Now the share taken by salaries has increased, leaving less for the other activities including research.

The ESF does not suggest what should be done. For the present it merely points out the dangers and urges countries to find their own solution.

The assembly approved a budget of 3.996m French francs for 1979. Among the activities it will finance are three new ones, two in the humanities—Byzantine and Chinese studies—and one covering the ESF's work in synchrotron radiation.

Ten member organisations are providing an additional 500,000 French francs for a feasibility study on building a European Synchrotron Radiation Facility. Such a machine would be distinguished from existing machines by its very high brightness, specially designed elements for each wavelength range, undulators which are very bright emitters and a beam time structure for time resolved studies. ESF hopes to complete its report within a year so a quick decision can be made whether to build the facility.

At the meeting of the Assembly, Sir Brian welcomed Dr John Goormaghtigh who takes over as Secretary-General from Dr F. Schneider in January 1980. Dr Goormaghtigh works in the social sciences. **Judy Redfearn**

Wiesner attacks increasing government regulation of US university research

INCREASING attempts to regulate university research have led to floundering relationships between the federal government and the academic community that have reached a "point of crisis", according to Dr Jerome B. Wiesner, science adviser to President Kennedy and now president of the Massachusetts Institute of Technology.

Speaking at a conference of research administrators in Washington last week, Dr Wiesner said that this could seriously curtail the effectiveness of the nation's major research universities, and that the increasing intrusion of the federal government into university affairs threatened their capacity to produce innovative ideas.

He singled out for particular attack proposals published earlier this year by the Office of Management and Budget to introduce significant changes in the accounting procedures—currently listed in the so-called Circular 21—by which universities compute how much they should be reimbursed for the direct and indirect costs of carrying out federally-sponsored research projects.

OMB has claimed that the revisions are aimed at rationalising the procedures used by different government agencies, and reducing the risks of the possible misuse of funds. Dr Wiesner, however, said that the proposed changes, which have been widely criticised by the research community, would weaken universities as institutions and reduce their capacity to conduct high quality research.

"There is no question, for example, that they limit in a destructive way reimbursement for indirect costs that are necessary and essential. There is no question that a number of the proposals are inequitable or administratively impracticable or both."

He called on President Carter to defer implementation of the revisions until a full study of the current problems faced by university research workers had been completed. "The proposed revisions to A-21 not only sacrifice flexibility but, far more significantly, they move in the direction of viewing universities in the same manner as commercial organisations and away from the concept of a partnership between the universities and the federal government."

Dr Weisner also criticised the recent decision of Congress, in passing the National Science Foundation's budget for the fiscal year 1979, to place a ceiling of $48,000—the maximum rate payable to Government scientists—on the salaries of university research scientists receiving university support.

"What this means is that Congress is limiting the reimbursability of salaries of the best faculty, the stars, the Nobel Prize winners, those people who make our institutions great. Universities will have to make up the difference, starting with an already substantial impact, and that's only the beginning," he said.

David Dickson

Few surprises in UNCSTD draft plan of action

A DRAFT plan of action has been published by the secretariat of the United Nations Conference on Science and Technology for Development (UNCSTD), scheduled to take place in Vienna next August. The plan will be discussed by the UN General Assembly in New York next week, and a revised version will then be considered by the conference's preparatory committee when it meets at the beginning of next year.

There are few surprises in the draft plan, which is essentially a synthesis of the various proposals made in the national and regional papers which have already been submitteed to the secretariat (a number of countries—perhaps most noticeably the United States—have yet to complete their national papers).

The draft plan of action has reduced the proposals to 201 separate recommendations grouped into two main sections: science and technology for development, and "institutional arrangements and new forms of international co-operation in the application of science and technology."

Popular themes include the need for codes of conduct covering both the transfer of technology and the behaviour of multinational companies, the need to improve scientific and technological information services and policy-making machineries, and concern about the effect of the "brain-drain" to developed countries. There are also suggestions for encouraging cooperation in research between developing countries, and ways of re-orienting educational systems towards national needs; and several countries ask for an emphasis on the development of appropriate technologies.

Other suggestions range from the proposal that developed countries set aside 0.5% of their gross national product for research related to Third World issues, to the suggestion that

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regional "multinational" companies be set up to break the monopoly of foreign transnationals in selected areas of interest to a particular region—something that could be achieved, it is suggested, by polling the resources of a group of countries in the region and by arrangements for market-sharing, as is now done by the Andean pact countries of South America.

The draft plan says little about basic science. One of the few explicit references is in paragraph 63, which says: "One developing country and one market economy country recommend the fostering of basic research as an important basis for indigenous scientific and technological development." But the same paragraph continues: "Several countries recommend a more utilitarian rather than an academic approach to the results of scientific research and underline the special importance of applied research and technological development for countries with limited resources."

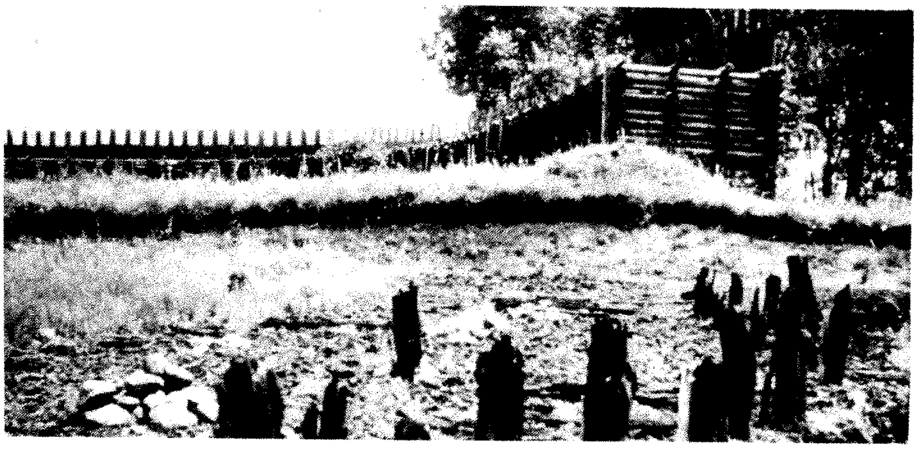
Nor is much attention given to the obstacles to applying science and technology to development although they had been stressed previously by the conference secretariat. The introduction to the plan of action admits that "the approach by most governments in the preparation of their national papers has been to deal implicitly with obstacles throughout their papers."

As a result, the secretary-general of the conference, Dr Frank Joao de Costa, says that he has adopted the same approach in the outline of the plan of action; and the introduction states that the number of obstacles treated explicitly "has necessarily been limited."

The two resulting paragraphs are vague and generalised. The first says merely that "systematic analysis and study" of such obstacles is a prerequisite for their elimination. And the second, in language somewhat typical of the whole document, states. "Many developing countries consider that the absence of an awareness of science and technology, often in the context of the social structure, cultural heritage, value system and religious traditions of a country may constitute an important obstacle to the application of science and technology."

David Dickson

Problems of preserving a 2,500-year-old township



Reconstruction of the palisade with original wood in the foreground. Below: applying the solution.

SINCE its discovery in 1933, Biskupin has posed a considerable problem to Polish archaeologists: how to excavate this prehistoric township whose timbers were preserved virtually intact in the peat without the immediate deterioration and decomposition of the finds once they are in contact with the air. A conference of wood conservation specialists, organised last September by the Polish State Archaeological Museum, which is responsible for conservation work at Biskupin, indicated, however, that during the last three years extremely satisfactory results have been attained at attempts at conservation *in situ*.

Biskupin, which dates from circa 550 B.C., was an island settlement of two-bedroomed wooden houses, arranged rectilinearly and enclosed by a palisade. Abandoned when the water-level of the lake rose, the houses collapsed and their timbers were preserved in the peat. The value of such a site for early iron-age archaeology is inestimable; however, until recently the problems of conserving the timber finds seemed insurmountable.

The new conservation programme at Biskupin uses a polyphenol resin to preserve the timber. The method is adapted from one introduced six years ago by Yurii Vikhrov, of Minsk, (Byelorussian SSR). In the Polish method the fluid is applied with a brush, and then cured by sunlight, temperature or catalyst hardening. Curing time depends on the conditions, but, using hot-air blowers, a typical figure would be 30 hours at 100 °C.

The resin penetrates the wood to a depth of 3 cm; this gives a mechanical strength that is quite sufficient for conservation; indeed, this method has already been used for the conservation of a whole mediaeval house at Brest-on-Bug. According to Teresa Stanczuk-Rozycka, head of the conservation laboratory of the State Archaeological Museum, the method, although expensive, nevertheless works out at only 20% of the cost of the method used in Sweden to conserve the timbers of the *Vasa*, and, unlike the Swedish method, is suitable for work *in situ*. Mrs Stanczuk-Rozycka further said that the September conference showed that the overall results of the method were extremely good, and that the programme of conservation would go forward.

At present, the conservation teams propose to work only on the remains of the gate and ramparts, excavated during the early digs, which are now under the greatest risk. With all future excavations, however, running conservation of the wood in parallel to the actual digging work will be carried out as a matter of routine.

And, since the actual technique of applying the fluid and, if necessary, the hot air blowers, is extremely simple, a large team of skilled conservationists will not be necessary. "Anyone can do it", Mrs Stanczuk-Rozycka, told *Nature*, "for example, students!"

Vera Rich

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Vietnam's scientists wait for books and equipment

AT THE HEIGHT of the Vietnam War, US Air Force chief General Curtis Lemay threatened to bomb North Vietnam "back into the Stone Age". Although American bombing never achieved this objective, it nonetheless destroyed a great many buildings, among them — factories, offices, hospitals and research laboratories. Today, in spite of extensive rebuilding office space is generally in very short supply, which means many scientists have to work in cramped conditions, particularly in Hanoi. In Ho Chi Minh City (formerly Saigon), things are not a great deal better, although it did emerge from the war less scarred.

Many of Vietnam's leading scientists have now moved to Ho Chi Minh City, under the auspices of the country's largest research establishment, the Vietnam Scientific Research Centre Laboratories of experimental biology, petrochemistry, and physics and chemistry of natural products are already established in the city. The latest addition is to be a laboratory of biochemistry and food chemistry, early next year.

One incentive for moving south is the prospect of working in some of the better-equipped food and pharmaceutical industries The great drawback is a shortage of scientific literature. According to one scientist, such material is in very short supply in Hanoi, but even this constitutes a "wealth much to envy" when compared with the south.

There have been reports of discrimination against scientists who worked for the south during the war. The latest information from Ho Chi Minh City however, suggests that northern scientists now in the south are making a major effort to bring their southern colleagues back into the fold. A major obstacle is the lack of available scientific equipment; the accommodation is there, but not the technical support, and it appears there is little the government can do to improve matters at present in the current economic conditions.

- The official British response to Vietnam is somewhat confusing. The Ministry of Overseas Development (ODM) and the Foreign and Commonwealth Office are known to be concerned with human rights in Vietnam, and new aid programmes are being refused until they are sure that no violations of rights are taking place. But in Vietnam itself, the British Ambassador has bought some £2,000 of much-needed scientific literature for biologists working at the Vietnam Scientific Research Centre in Hanoi He is apparently allocating a further £2,000 for books next year.

- Back in Britain, neither the Royal Society nor the British Council appears to be doing much to promote contacts with Vietnamese scientists, despite discussions earlier this year about inviting one or more Vietnamese scientists to the UK. A Royal Society official said it was "into China at the moment". But a leading British scientist has urged the Society not to abandon its interest in the scientists of Vietnam who are in urgent need of assistance. The Royal Society, he said, could do much to help, simply by making and maintaining contact with the Vietnamese.

Alastair Hay

India gets ready to tune in to its satellite

THE building of seven ground receiving stations has begun as the first stage in preparing for the Indian National Satellite (INSAT) programme. The stations will be linked next March with a fractional transponder aboard an INTELSAT satellite above the Indian Ocean.

The INSAT project, costing six crore, is planned to provide remote areas with communications. By the time INSAT-I is launched in 1981, 28 more earth stations will be built all over the country.

INSAT-I is a multipurpose domestic communications satellite with a seven year lifetime to be built by the US Ford Aerospace Corporation. It will provide nation-wide direct television broadcasting to community receivers in rural areas, and continuous meteorological data in the visible and infrared.

Of the 35 ground stations to be built, six will be mobile, four by road and two by air. The one at New Delhi will be the biggest, acting as the control and monitoring station for the others. For meteorological studies about 110 unmanned data collection platforms will be installed at inaccessible spots. INSAT-I will give a cloud picture over the Indian Ocean, the Arabian Sea and Bay of Bengal every half hour.

As well as the Department of Space, other agencies like Posts and Telegraphs, Indian Meteorogical Department and the Ministry of Information and Broadcasting will be responsible for the establishment and operation of the INSAT network Most of the equipment needed to build the first seven earth stations will be imported, but Indian-made equipment will be used for subsequent stations The total INSAT project will cost Rs 175 crore with a recurring expenditure of Rs 11 crores per annum. ☐

Third World seeks low frequency broadcasting

THE non-aligned countries should seek a suitable frequency for direct sound broadcasting via satellite from the World Administrative Radio Conference (WARC) when it meets next year. This was decided at a three day conference on "Satellite in Broadcasting" among the non-aligned countries recently held in New Delhi.

According to experts at the conference direct sound broadcasting via satellite would enable the member-countries to use relatively inexpensive and mobile receivers The Indian Information and Broadcasting Minister Shri L. K. Advani said that high costs and the need for high-level expertise need not deter the member-countries from using satellites for communications Costs could be reduced through collective efforts, he said, and the technical expertise was already available in the developing world.

The purpose of the conference was to prepare a comprehensive report on the technical, economic, legal and political aspects of satellite broadcasting which will be presented at the forthcoming meeting in Tanzania of the Committee for Cooperation among the Broadcasting Organisations of Non-aligned Countries.

The experts felt that the selection of a particular mode of TV or radio transmission must depend on local conditions. They outlined a way of choosing the best mode taking into account the geography and available infrastructure of the individual country. They also decided to work out a way of exchanging TV and radio programmes among the member countries currently using the INTELSAT system.

Directly linking the domestic satellist of non-aligned countries is not yet possible because they transmit at high frequencies. The conference therefore agreed to urge the WARC to allot a frequency lower than 54 GHz —the lowest frequency band so far used. India is likely to ask for a long wave frequency because of the work she has done on long wave frequency propagation technology for low cost and long range broadcasts.

Dilip M Salwi

US carcinogen control threatened by costs

THE US Department of Labour's Occupational Safety and Health Administration (OSHA) seems to be losing the fierce battle that it has put up against being required to carry out detailed cost-benefit analyses of attempts to regulate occupational exposure to toxic substances

OSHA director Dr Eula Bingham, previously professor of environmental health at the University of Cincinnati Medical School, remains adamant that such analysis is not required by the Occupational Safety and Health Act of 1969, beyond ensuring that no action threatens the overall survival of a particular industry.

OSHA is far from giving in to the attacks of those who claim that further regulation is merely aggravating the country's inflation problems. This week, for example, the Department of Labour was expected to announce a new standard for occupational exposure to lead of 50 micrograms per cubic metre of air—four times lower than existing permitted levels—despite its own studies showing that this will have a substantial economic impact on the lead-storage battery industry

However, two recent events indicate the increasing pressure on OSHA to act not just on scientific evidence—or lack of evidence as the case may be—to protect workers' health but also to consider the economic implications.

The first is a statement from the Regulatory Analysis and Review Group, an offspring of the Council on Wage and Price Stability widely distrusted by environmental and labour groups In its comment on OSHA's proposed scheme for classifying and regulating chemical carcinogens, the review group echoed opposition to the scheme by the US chemical industry, criticised its 'inflexilibity' and claimed that the cost of implementing it would be "potentially quite substantial, well in excess of $1 billion."

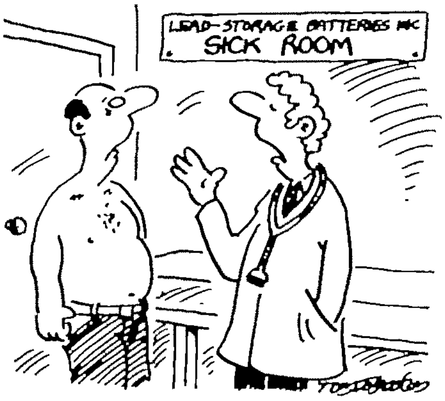
Similar pressures are also beginning to come through the courts. Last month, a federal appeals court in New Orleans set aside proposed new benzene exposure rules proposed by OSHA in June, on the grounds that the agency had failed to demonstrate a "reasonable relationship" between anticipated benefits and costs.

OSHA has already indicated that it will appeal against the court's ruling, a significant departure from earlier legal decisions which had accepted that such cost-benefit analysis was not necessary. But it accepts that if the ruling stands, then it would be required to shift its strategy; "if one has enough interpretations that this is demanded, then one would do it", Dr Bingham said.

The agency's reluctance to engage in such analysis seems based on three fears about how it might undermine OSHA's mandated efforts to protect the health of US workers. The first is that a requirement to carry out detailed cost-benefit studies would overburden an understaffed agency.

The second fear is that, as far as information on costs is concerned, OSHA would have to rely heavily on data provided by the industry itself, which it feels might be overstated

Thirdly there is a feeling that occupational health is an area in which scientific uncertainties are so high that any attempt to place definitive values on degrees of risk, etc., would merely disguise how such judgments reflect broader social values, and that these could be better dealt with if openly acknowledged.



"You're suffering from a bad case of Federal Appeals Court decisions, aggravated by inflation problems!"

"There is in scientific terms no evidence of a 'threshold'. But there is evidence that as you reduce the exposures, you are reducing the risk. If there are a few fibres or molecules left, theoretically someone somewhere might get cancer from it. At that point a social decision has to be made," according to Dr Irving J. Selikoff director of environmental sciences, Mount Sinai Hospital, New York

The problems caused by the scientific uncertainties were illustrated by the public controversy last month over a statement released by the National Cancer Institute, the National Institute of Environmental Health Sciences, and the National Institute for Occupational Safety and Health, claiming that up to 20% of all cancer deaths might be attributable to occupational exposure

Erroneously described by the US media as the results of a "new federal study", and frequently hardened to the claim that 20% of cancers were attributable to such exposure, the report was in fact a position paper put together rather hurriedly to meet OSHA's deadline for comments on its carcinogen proposals—and was given premature publicity when Labour Secretary Ray Marshall quoted extracts at a trade union conference.

The main message of the statement is that earlier estimates suggesting one to five per cent of cancers can be attributed to occupational exposure appear to be "unrealistically low"

However, the American Industrial Health Council, an industry-sponsored group which has been leading the attack on OSHA's carcinogen proposals, criticised the 20% figure as based on "unwarranted speculation".

Emphasising the council's support for the one to five per cent estimate, Dr Fred Hoerger, chairman of the AIHC's scientific committee, said that "the predicted massive increase in cancer due to occupational exposure in the study is not supported by current knowledge of cancer rates, worker exposures, or numbers of workers exposed".

The debate has demonstrated the gulf that exists between the scientifically conceivable (represented by the NCI position), and the scientifically demonstrable (on which AIHC argues regulation should be based).

It is a gulf which OSHA feels would be prematurely foreclosed by translating the debate into one of costs against benefits, given the problems of adequately defining either. Industry has been impressing on the president's economic advisers that, with rapidly rising production costs, this is the only reasonable basis for legislation.

OSHA has so far taken a firm stand against being required to adopt industry's perspective. And it has suffered virulent attacks, such as the American Conservative Union's "Stop OSHA" campaign, for doing so. But it is not yet prepared to throw in the towel

David Dickson

Correction

On page 108 of last week's issue a small error may have caused some confusion in interpreting the UK's proposed new guidelines for genetic manipulation. In the third column, item 4, the experiments referred to are those listed under the third item in the box on pages 106 and 107, that is those in Category I* "for which at present there is no substantial evidence of risk. The chief candidates of this kind are the experiments in which *E coli* genes are cloned in *E. coli* organisms".

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news in brief

● **US Academy confirms saccharin as potential carcinogen:** THE US National Academy of Sciences has confirmed that the artificial sweetener saccharin can cause cancer in laboratory animals, and that it is probably a 'low potency' carcinogen in humans.

In a report prepared at the request of Congress, which last year imposed an 18-month moratorium on a proposed ban of saccharin by the Food and Drug Administration, an academy review panel says that "even low risks applied to a large number of exposed persons may lead to public health concerns".

The panel says that saccharin may also enhance the potency of other cancer-causing agents, and suggests that it may in fact be more dangerous as a 'promoter' of other carcinogens than as a carcinogen itself.

The panel also expresses concern at the increasing quantities of saccharin being consumed by young children, mostly in diet soft drinks. The length of time which cancer takes to develop could mean that such children have a magnified chance of developing cancer later in life, the panel says in its report.

● **Reprocessing plant still closed:** Employees at the Windscale reprocessing plant are still waiting for British Nuclear Fuels Ltd and the Nuclear Installations Inspectorate to decide when to re-start the reprocessing of nuclear waste. The plant was closed two weeks ago after a potentially explosive build-up of hydrogen in one of the silos where magnox cladding material is stored. In a reply to questions from the conservation group Friends of the Earth, the Department of Energy said workers were not in danger from the release of radioactivity from the silo, but reprocessing would not restart until an investigation revealed the cause of the build-up of hydrogen and if any measures needed to be taken to prevent a recurrence. A number of questions remain to be answered. For example, why, after 15 years of storing magnox in this manner, has the build-up of hydrogen not occurred before? A spokesman for British Nuclear Fuels told *Nature* that they wanted to be sure they understood what had happened before restarting reprocessing.

● **US judge claims low levels of PBBs "not toxic".** A county circuit court judge in Michigan has ruled that the low levels of polybrominated biphenyls (PBBs) to which cattle in the state were exposed after fire retardant had been mistakenly mixed with animal feed, were "not toxic". As a result, he dismissed a claim for damages issued by a Michigan farmer who slaughtered his herd on realising that they had eaten contaminated feed provided unknowingly by the Farm Bureau Services, a farmers' cooperative, in 1973. In a 177-page opinion handed down after the longest trial in state history, Judge William Peterson said that the animals had been slaughtered needlessly because of public fear aroused by "incompetent and dishonest" scientists, veterinarians and lawyers who, he claimed, were trying to capitalise on the disaster.

Fears about the potential carcinogenicity of PBB have been raised because all other halogenated aromatic hydrocarbons tested to date have turned out to be carcinogenic. Research on PBB is now being carried out at the National Cancer Institute in Bethesda, Maryland. However, referring to the arguments of those who had warned of the potential long-term hazards of low-level exposure to PBBs, Judge Peterson said: "Professionals have forsaken objectivity and their usual standards of inquiry to accept unquestioningly, as a basis for their expressed opinions, reported facts that were not factual."

● **$1.5 million to erect statue of Einstein:** The US National Academy of Sciences announced this week that it is to erect a statue of Albert Einstein, three times life-size in the academy's grounds on Washington's Constitution Avenue. The statue, which will be unveiled next spring in a ceremony honouring the centennial of Einstein's birth, will be carried out by Robert Burks, who sculpted a portrait head of the physicist in 1953. The cost of the statue, the base and the ground preparation will be more than $1.5 million. A fund-raising campaign is being organised by the academy among the US technical community.

Announcing the plans for the statue this week, Dr Philip Handler, president of the NAS, said: "It is our hope that this statue will serve several public purposes—as a tribute to the enormous analytical and creative powers of this unique scientist to whom we owe so much, as an evocation of his profound spirituality and his concern for mankind, as a reminder that Einstein found in this country refuge from tyranny, and finally as an appreciation of the accepted place of science in our civilisation."

Meanwhile, in Europe, two interdisciplinary scientific meetings in Munich and Ulm marked the beginning of an international series of meetings to be held throughout 1979 to commemorate Einstein's birth on March 18, 1879. At the meeting in Ulm, Paul Dirac, who worked with Einstein at Cambridge and Princetown and who was awarded the Nobel Prize for physics in 1933, paid a tribute to "the excellence of Einstein's theory of gravitation".

● **Indian shuffle:** The new Secretary in the Indian Department of Science and Technology is Professor M. G. K. Menon. He will also serve as director-general of the Council of Scientific and Industrial Research. These posts became vacant with the departure of Dr A. Ramachandran to head the UN Centre on Habitat in Nairobi. Professor Menon is at present chairman of the Electronics Commission and secretary to the Department of Electronics and was for several years scientific adviser to the Minister of Defence. Well-known in the international science policy world, he has served as a member of the UN Secretary-General's Advisory Committee on the Application of Science and Technology to Development. His research in the fields of cosmic rays and elementary particles was largely done at the Tata Institute of Fundamental Research, of which he was director from 1966 to 1975

● **Britain joins in China exchange:** China is continuing to expand its scientific cooperation with western countries. Up to 100 Chinese research workers are to come to UK scientific laboratories for periods of about four years, under the terms of an agreement between the Royal Society and Mr Hu Ke-shih, vice-president of the Chinese Academy of Sciences, which was signed on 10 November. Mr Hu was leader of a delegation of scientists and Academy administrators who have just concluded a three-week visit to the United Kingdom as guests of the Royal Society. The group visited many university departments and research establishments such as the Rutherford Laboratory at Chilton and the Imperial Cancer Research Fund Laboratories in London. The agreement comes soon after a similar deal to exchange post-graduate students between China and the United States.

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Should scientists attend the ICUS?

● When the third International Conference on the Unity of Sciences (ICUS) was held in London in November 1974, scientists were made aware of the link between the International Cultural Foundation, the conference organisers, and Mr Sun Myung Moon, the head of the much criticised Unification Church (Nature 251, 661; 1974). Since then, scientists have been divided over whether to attend subsequent sessions of ICUS. Here, Professor N. Kurti of the Department of Engineering Science, Oxford University explains why he feels justified in attending them.

THE seventh International Conference on the Unity of Sciences (ICUS VII) will take place in Boston, Mass., 24–26 November, 1978. No other series of scientific conferences has engendered so much passion, largely because these conferences have been financed and run by the International Cultural Foundation, one of the 50-odd organisations founded and presided over by Mr Sun Myung Moon, the wealthy, and passionately anti-communist South-Korean industrialist and businessman.

Wealth, strong political bias, success in business are not normally considered unacceptable characteristics in sponsors of conferences, but Mr Moon has one further attribute which is at the bottom of the animosity which the activities of the Moon-organisation create: he is the founder and head of the Unification Church which has hundreds of thousands of followers in South Korea and Japan—though its active membership in Western Europe and North America is probably well below 15,000 He is regarded by his followers as the new Messiah called by God to finish the work begun by Jesus Christ—and to my knowledge has never made an explicit disclaimer.

The Unification Church has been strongly attacked for its forceful proselytising activities and accused of brainwashing young people and alienating them from their families. It has many aspects which I find distasteful. The following excerpts from an interview Mr Moon gave to American Newsweek International are revealing: "God has hand-picked me" (The basis of an intense personality-cult). "The line-up in Panmunjon is like the line-up between the heavenly world and the satanic world" (President Park's South Korea seems to me a strange sort of heaven). "If one of my disciples denounces his parents he is not really my disciple" (How many of the Moon-disciples who did break with their families were expelled from the Church?).

It is not surprising that the following two questions should have been asked often: can a conference sponsored by a militant and politically motivated organisation which many regard as a danger, not only to the spiritual well-being of young people, but to the structure of society as a whole, do its work in an unbiased, objective fashion without being influenced by the views and beliefs of its sponsors? Are the scientists, among them many world-famous figures, allowing themselves to be used as propaganda material for the Unification Church? Having attended five of the six conferences held so far I shall try to answer these questions.

The first conference, organised by Mr Edward Haskell, author of the book *FULL CIRCLE, The Moral Force of Unified Science,* and held in New York in November 1972 was a dismal affair This is not surprising, since the above book, which formed the basis of the meeting, contained such choice proposals as periodic tables for the "Plant Kingdom", the "Animal Kingdom", "Human Society", etc. After all if science is "unified" then what goes for chemistry goes for other disciplines!

Such was my disillusionment with this first ICUS that in the summer of 1973 I declined an invitation to attend the second conference in Japan. I did the same when I was asked a year later to attend ICUS III to be held at the Royal Lancaster Hotel in London. However when I noticed that several of my colleagues for whom I had great respect were associated with the conference and had helped to shape its programme, I accepted, and did not regret it. ICUS III, together with ICUS IV held in New York in 1975, were, in my view, the best of this series.

One might well ask whether anything worthwhile can come out of conferences devoted to the unity of sciences—science being used in the wider sense of the word, equivalent to the German *Wissenschaft* or the Russian *Nauk*. Those who search for or want to believe in a 'unity of sciences' are often tempted to transfer concepts appropriate to one discipline to a totally different one and proclaim the result as a proof of 'unity'. Mr Haskell's periodic tables for botany, zoology, sociology are examples, so are Mr Moon's speculations about the origins of energy given in his address to ICUS V: "Without there first being a subject and an object pair, it is impossible to produce energy. That is to say, the relationship of a subject and object is indispensable as in the case of an atom where the proton (which is the subject) and the electron (which is the object) must both be present before beginning to interact". Hence "Energy is the phenomenon resulting from the process of the two (subject and object pair) becoming one". The rest follows: happiness is the result of "mind and body being united in one". Illness is the result "of the subject and object elements of the body failing to attain unity and harmony"—the medicine prescribed by the doctor "restores the harmonious unity".

If 'unity of sciences' is an ill-chosen aim of this series of conferences, the choice of the titles of the individual conferences was equally poor. What can one make of 'science and absolute values' (ICUS III), 'the centrality of science and absolute values' (ICUS IV), 'the search for absolute values: harmony among the sciences' (ICUS V)? But fortunately conferences are not determined by titles and slogans but by the quality of the participants and their interests, and even meaningless clichés can lead to useful conclusions and the crystallisation of ideas.

The following examples may illustrate this point At ICUS III in London one of the four committees spent two days on lectures and discussions under the general heading 'the idea of the university in a troubled world' One of the papers had the daunting title 'international education: a search for cultural and conceptual isomorphisms', but fortunately it had as an explanatory motto a quotation from Mark Twain:

> "I went next to the Secretary of War . . . I said there was nothing so convincing to an Indian as a general massacre. If he could not approve of the massacre, I said the next surest thing for an Indian was soap and education Soap and education are not as sudden as a massacre, but they are more deadly in the long run; because a half-massacred Indian may recover, but if you educate him and wash him, it is bound to finish him some time or other. It undermines his constitution; it strikes at the foundation of his being."

There was a lively discussion by philosophers, historians, economists, physicists, etc., on the question of whether or to what extent one civilisation should impose its system of education on another. As one participant said: "Is the educational system developed in Europe during many centuries fruitful for the Bantu child in a surroundings of tribe-togetherness?" Another paper under the same general heading was entitled 'cognitive limitations and public decision making' and led to an examination of the relative roles of intuition and statistical analysis in decision making

As in ICUS III so in ICUS IV (New

York) the interaction between developed and developing countries came under scrutiny. Thus the Committee which discussed 'the university, research institutions and society' returned again and again to that particular question, as the following examples show:

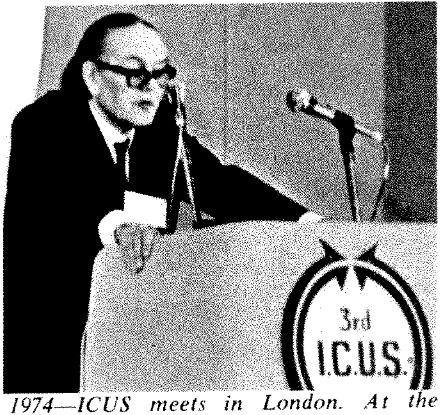
- Should universities in developing countries follow the principle enunciated in 1896, "only the best is good enough for Africa", or, should they eschew "excellence" in favour of "relevance" and be more attuned to local needs and aspirations?
- There seemed to be a need to scale down well-established technologies to suit developing countries, so that they are not forced to buy needlessly large plant, because nothing smaller was available.
- How can developing countries be persuaded to accept "intermediate technology" and not feel that they are being "fobbed off with the second best"?

I believe that even the severest critics of these conferences, agreed that the lectures and discussions were free of constraints, and that the proceedings of the conferences were not censored at all. The best proof can be found in Professor A. J. P. Martin's controversial lecture given at the closing session of ICUS III. One of his points was that there are too many people around, and that the population of the world, should be reduced by a factor 10. He proposed, for instance that the prison population should be reduced by submitting the inmates to carefully dosed X ray treatment which drastically shortens the expectation of life. These and similar suggestions, for example about selective breeding of humans, cloning etc., were delivered in deadly earnest, and I just could not judge how much of it was meant seriously and how much was *"pour epater les bourgeois"*, or rather *"les moonies"*. But what I find important is that Professor Martin's lecture, all of which was anathema to Unification Church tenets was printed in full in the proceedings.

It cannot be asserted that what was said at the unity of sciences conferences could generally be used in support of the Unification Church, but it is arguable that who said it could be of propaganda value. Indeed there have always been many Nobel-Laureates among the participants—at ICUS III in London 25 out of 140—and most ICUS Chairmen were Nobel Laureates: Lord Adrian (ICUS III), Professor R. S. Mulliken (ICUS IV), Sir John Eccles (ICUS V and VI), Professor E. P. Wigner (ICUS VII).

It is often said that the presence of so many Nobel Laureates and other well known scientists at the conference could be used to enhance the "respectability" of the Unification Church, and to neutralise the attacks made on it. Such fears are supported by the continuous tape-recording, filming and photographing at these conferences. But the remedy is often in the hands of the participants. When after ICUS VI the organisers were compiling a little glossy brochure about these conferences to lessen the effect of hostile press comments, participants who were to be quoted were asked for their agreement. I thanked the organisers for their courtesy, said that I regarded these conferences as *bona fide* scientific affairs which should be judged by the contents of the published conference reports and not by selected remarks of participants and others. My request not to be quoted was complied with.

To what extent are these conferences truly "international"? In 1975 I pointed out to the organisers that a conference to which people from the Communist countries are apparently not invited and which, while having quite a few participants from Israel, has none from Arab countries, could not be called "international". I made certain concrete suggestions and since then there is representation, albeit small, of both Arab and Communist countries.



1974—ICUS meets in London. At the microphone: Professor Archer Martin.

It is sometimes said that these conferences are used to make propaganda for the Unification Church and that the programme is arranged and the speakers chosen to suit the Church's book. My personal experience does not bear this out. As a member of the International Advisory Committee I frequently suggested speakers and participants whose religious and political ideas were far removed from those of the Unification Church, and yet my suggestions were invariably accepted. Of course many of the participants, among them scientists of considerable reputation, admire Mr Moon and the Church, but this does not interfere with their scientific judgment and in fact the Church, and for that matter Mr Moon, are hardly mentioned if at all.

I shall now answer the two questions I posed at the beginning of the article. The ICUS are *bona fide* scientific conferences free of political and religious bias. Not everyone may agree with the choice of topics, but this is not surprising for conferences which draw their participants from disciplines spanning most intellectual activities.

The second question, namely to what extent one's participation at ICUS furthers the aims of the Unification Church falls into three parts.

Has it any propaganda value? The films and recordings made at ICUS are, I believe, shown at Unification Church centres and functions, and it could be that the Church's aspirants and disciples are gullible enough to believe that the conference participants are *ipso facto* supporters of their Church and that the Nobel Laureates photographed with Mr Moon are his followers. Against this must be said that the conferences give ample opportunity for talking to the press, radio and to members of the Church, and many participants make it clear on such occasions that they have nothing to do with the Unification Church—perhaps even making critical remarks about it. For them ICUS is simply a multi-disciplinary scientific gathering.

Does acceptance of the fare and full board put one under an obligation to the International Cultural Foundation and to the Unification Church and colour one's attitude to them? If this were so, then scientists who regularly receive funds from many sources would be among the most diversely bribed members of the community owing allegiance to a multitude of organisations. Is the integrity of scientists really rated so low?

Can attendance at a conference sponsored by or connected with a religious or political organisation of whose activities one disapproves be morally justified? This is similar to the perennial question: should one attend conferences in countries with whose political system one disagrees, or which persecutes some of its citizens? There are those who say "No". For instance, there were some French people who even several years after the German occupation could not bring themselves to set foot on German soil.

But others say that adherence to such rigid principles, would make international scientific exchanges and collaboration impossible. One must follow one's conscience, and I personally have no moral qualms about attending an interesting and useful conference provided I am satisfied that in doing so I am not furthering aims or activities which are regarded as undesirable or even harmful. □

correspondence

The fate of the Taj Mahal

SIR,—M. K. Agarwal has made a plea (2 November, page 10) that India should go ahead with the construction of the proposed oil refinery near the Taj Mahal and asks whether a poor nation can afford the luxury of keeping its monuments in their pristine beauty while it cannot provide a minimum for its people. While the intentions are laudable, I would like to contest the rationale.

If the shifting of the proposed site of the refinery by a few hundred miles is really going to affect the economic development of India to a significant degree, Mr Agarwal might indeed have a case. If there are scores of other marble monuments across the country which are all threatened by projects vital to reduce human misery, he may again be justified in criticising the efforts to save the Taj Mahal from corrosion, as one cannot go around cancelling all development schemes.

While changing the site of the refinery will only cause a bit of a headache for the planners and the bureaucrats, I cannot see much harm even in completely dropping the proposal. Looking through India's record since independence, no sincere observer can escape the fact that despite her impressive technological progress, the percentage of the population under the starvation line has remained around 60%. In absolute numbers, poverty has only increased.

The massive nuclear reactors, steel factories, refineries and the rest have benefited mostly the affluent and the urban middle-class. The majority of poor rural India, which should be the primary concern of any development project, has hardly gained from the industrial growth. In fact a case can be made that this western style technological progress has done more harm directly by increasing the stranglehold of the big industrialists, and indirectly by diverting valuable money from investment in the rural sector and in appropriate, labour-intensive, small scale technology. One could also argue that without fundamental social changes and agrarian reforms, any industrial progress in India can only be harmful.

Rather than being an economic liability, the Taj Mahal has always brought enormous foreign exchange to India. No tourist leaves the country without visiting Agra. Even commercially, it would be wiser to back the Taj Mahal rather than the refinery. Under these circumstances, the effort to save it from corrosion is hardly a luxury for a poor country.

If the construction of the oil installation goes ahead and the pollution does damage the monument irreparably, posterity will never condone this generation for sacrificing one of mankind's great architectural wonders for a very dubious (perhaps despicable) economic reason.

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Discussing values and judgments

SIR,—I was most interested in your editorial comments of 20 July and feel constrained to address some of the inconsistencies they contain. In the first place, the separation of scientists from "the others" in terms of their ability to discuss values and human judgments and to deal with "convergent" rather than "divergent" problems seems to be both misleading and patently false.

During my years at university, I was constantly made aware of the barriers erected between the arts and sciences and was frequently told that "you are unable to understand these issues because your scientific training hasn't given you the necessary foundations for understanding general cultural and value systems." Rubbish! What I *have* observed during my years in university and subsequently is that non-scientifically trained graduates tend, if anything, to be a bit more dogmatic in their beliefs than scientists and are often unable to carry on philosophical discussions in a logical and consistent manner.

My education prepared me to make value judgments and the scientific method has fitted me admirably to address open-ended problems in a reasonable way.

All of your analysis seems to address reaction to recent evaluations of the Windscale inquiry and, specifically, the remarks of Professor David Pearce. You make further reference to the fact that the nuclear industry appears unwilling to discuss values—including the fear of nuclear weapons proliferation. Having chosen to "leave" such speculation (with which term you correctly indentified your ramblings), I should like to judge on its own merit your recommendation that school children be politically educated by requiring study projects linked with technological issues, such as the need for fast breeder reactors. In this way, you suggest, the humanist and the scientist (again, this false separation of the two aspects of each of us) can learn from one another. Assuming that you are referring to pre-university education, I very much doubt that your desired goals will be reached, since it is unlikely that such students are well enough prepared for the kind of in-depth study required to achieve these ends.

I should like to make further comment concerning the remarks of David Pearce in the following discussion of the values which are at the root of the nuclear debate. His statement proposing that opposition to nuclear power derives from differences in value systems strikes at the heart of this controversy; however, as I read them, his comments suggest that the "image of infinite credibility" is a cloak worn only by the nuclear industry and that they, so deceiving themselves, stand in the way of clear discussion of the values which lie beneath the issues discussed at Windscale. In a way this is rather humorous for, from my position on the other side of the controversy from that taken by David Pearce, it appears that this cloak is being worn by opponents of nuclear power development. I was glad to see him admit that there were many "men of reason" characterising the pro-nuclear stance but the impression given in his article was that this was the exception rather than the rule. With this same proviso, I could change the term 'industry' into the term 'environmentalist' and make the entire article read very much to my liking—perhaps proving, as Pearce suggests, that the debate has fallen to a very low level indeed.

A clue to the problem (which is raised by discussions such as the one presented by Pearce) is revealed when one considers the 'problem' of defining public accountability for decisions made in a social context. Institutions must be modified to accommodate other points of view, states Pearce, with the thinly-veiled threat that failure to do so will result in conflict (presumably including physical violence). I suggest that the real problem in the nuclear debate is the fact that institutions have been modified to the point where everyone, no matter where his expertise lies, presumes himself fully-qualified to speak on every issue and, for this reason, totally irrational points of view are given prominence simply because of their sensational value in the public eye.

Heaven help our society if orderly inquiry into questions such as that recently carried out at Windscale is replaced by mob rule because people such as David Pearce are unprepared to accept defeat when their values are weighed in duly-constituted public inquiry and rejected. Justice Parkers' report seemed a well-reasoned and impartial survey of the many questions brought up at the inquiry and the fact that he dismissed out of hand a number of alternative value systems proposed by some of the intervenors is not a result of his failure to understand but simply a judgment based on his understanding of the will of the people of Great Britain as expressed through past legal decisions and acts passed by their elected representatives in Parliament.

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Food production has kept up

SIR,—I was disappointed to see in your pages (26 October, page 698) the myth that there has lately been an "inexorable increase in the world's population without a commensurate increase in food supplies". FAO data show that during the past two decades of rapid population growth, while population has grown at 2% per annum food production has grown by an average 2.8% per annum. These results have been discussed in detail in *World Futures* (C. Freeman and M. Jahoda (eds), Martin Robertson, London; 1978).

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news and views

Powder metallurgy: new techniques, new applications

from Robert W. Cahn

CERAMIC objects—crockery, fine porcelain, furnace tubes, insulators—are made by starting from a powder, binding it (with water or adhesive), sometimes pressing the mass and then firing. The bulk of the powder never melts (though part of it may); by a series of physical processes including bulk and interface diffusion, the particles weld together, or sinter, and the pores between them close up. There is really no alternative, because most ceramics are too refractory to melt and, being brittle, the power particles cannot be squeezed together.

The idea of adapting sintering methods to metals seemed eccentric when it was first tried in Austria early this century, and the German term 'Metallkeramik' showed the origin of powder metallurgy as a variant of ceramic procedures. From the beginning, the attraction of powder metallurgy has been economy : a fairly complex shape can be made quickly and cheaply, without the problem of solute segregation inseparable from all forms of casting. In some special instances, powder metallurgy can create a product that cannot be matched by traditional means : the manufacture of long-lasting incandescent lamp filaments is the prime instance. (The progressive improvement of electric lamps, largely a matter of metallurgical ingenuity, is a fascinating chapter in the history of metallurgy). Apart from such special cases, powder metallurgy has generally been restricted to unsophisticated products, subject to modest demands.

All this is in the throes of change, and powder-metallurgical methods are beginning to be applied to demanding functions such as gas turbine disks and highly stressed airframe components. The reason is to be found largely in two very recent technical developments —rapid solidification processing and hot isostatic pressing.

Rapid solidification processing (RSP) is an American industrial development stemming directly from the techniques of splat-quenching of alloy ribbons from the melt (see *Nature* **252**, 100; 1974). An alloy melt is atomised and sprayed through a helium atmosphere and the fine droplets, usually in the range 25–100 μm diameter, are frozen very rapidly; the exact cooling rate is subject to many experimental variables, but an average rate of $10^6\,^{\circ}\mathrm{C\,s^{-1}}$ is quite accessible. The particles are then pressed and sintered and may subsequently be extruded or wrought in other ways. This technique of making final products has the advantages that macrosegregation is entirely eliminated, large amounts of solute can be held in metastable equilibrium which makes possible new levels of precipitation-hardening, and the consolidated product can have very fine grain sizes which in turn leads to great ductility at ambient temperature—even to superplastic behaviour.

The method is being applied to superalloys for jet engine parts, titanium alloys for airframe components and to aluminium alloys. It has been difficult to discover much about these developments, many of them shrouded in industrial secrecy—but publication has now begun. Much information about both powder quenching and consolidation is to be found in the proceedings of a conference on Rapid Solidification Processing.* The other up-to-date source is a review article by N. J. Grant, presented at the Third International Conference on Rapidly Quenched Metals, Sussex University, in July 1978; this will be published in the *Proceedings* by the Metals Society, London, in December 1978.

Hot isostatic pressing (HIP) is a new technique, pioneered in Sweden, with even wider potential. A chamber (which may be a fair part of a cubic metre) is subjected to high temperature—it can be well above 1,000 °C—and high uniform (isostatic) pressure—typically 1 kbar—transmitted by argon. The maintenance of such temperatures and pressures in such large volumes represents a technological achievement of a high order, with correspondingly high demands on safety precautions : in the early days of HIP an operator was suffocated in a sea of escaped argon.

Specimens for HIP consist of loose powder contained in a sealed evacuated can of metal foil or in a shaped die of glass or steel, and the process converts them in one operation into pore-free solids. Ordinary sintering of metals, without pressure, will not reduce porosity below 3–4%, and removal of that small porosity leads to a large improvement in strength, ductility, toughness and fatigue resistance at room temperature. A related function of HIP is its use for 'rejuvenation' of turbine blades. Creep of a cast blade in service generates porosity at grain boundaries, which limits creep life; HIP can entirely remove this porosity and thus effectively confer on the blade a new lease of life. This method is just beginning to be used in practice. A further development, in America, is the 'near-net shape' process. A shaped thick die of glass or steel is used to contain the powder : at high temperature and pressure, the die behaves as a quasi-Newtonian fluid and transmits hydrostatic pressure. Complex airframe components can be made from powder —which may have been made by RSP—directly as 100% dense shapes which require only a minimum of machining.

The combination of RSP and HIPping (the neologistic verb is firmly entrenched in America) offers, for the first time in many years, some prospect of enhancing the service temperature of turbine blades. This is because the problem of macrosegregation in castings has strictly limited the permissible alloy content of superalloys; RSP has removed this limitation and a new range of alloy compositions is now accessible. The main problem is that the fine grain size produced by RSP, desirable for applications at ambient temperature, is quite unacceptable for turbine blades—which must have very coarse grains. There are indications

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*Conference on Rapid Solidification Processing, Principles and Techniques, November 1977, Reston, Virginia, proceedings now published by Claitor's Publishing Division, Baton Rouge, Louisiana.

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that it may be possible to achieve such grains by recrystallisation after RSP followed by HIP, without loss of the precipitation-hardened microstructure that confers hot strength. In this connection, current theoretical work aimed at identifying the plastic processes that enable pore closure during HIP-ping may prove important, because the feasibility of recrystallisation is closely linked to the amount and nature of plastic deformation previously undergone by the metal. Activity in this field is very intense.

Yet another potentially very valuable use for HIP has just been proposed by U. Engel and H. Hübner of Erlangen, Germany (*J. Mater. Sci.* **13**, 2003; 1978). They HIP-ped, at 1 kbar and 1,260–1,360 °C, samples of cemented carbide (tungsten carbide particles cemented by films of metallic cobalt, made by conventional sintering combined with partial melting). These very brittle materials are used as tips for lathe tools subject to exceptionally severe demands. It was found that, after HIP-ping, all residual pores were completely eliminated, being filled by cobalt, which is itself not brittle. It appears that under HIP conditions the cobalt binder can flow freely between the carbide grains. Sophisticated methods of fracture testing were employed to examine the effect of HIP-ping on the resistance to brittle fracture, and it was found by statistical (Weibull) analysis that the mean fracture strength could be doubled, with a concomitant reduction in scatter of strength values. A critical comparison of observed flaw sizes with those computed from measured fracture strengths proved that the specimens were behaving as ideal brittle, flaw-limited solids both before and after HIP-ping. This very careful study indicates that HIP of cemented carbides should greatly increase the performance and life of lathe tools; moreover this should make economic sense as a production treatment, since cemented carbides are materials of high specific value. □

Megagauss magnetic fields in laser-produced plasmas

from M. H. Key

A recent paper by Raven, Willi and Rumsby (*Phys. Rev. Lett.* **14**, 554; 1978) describes the observation of an intense (1.8 MG) toroidal magnetic field surrounding the 30 μm focal spot of a pulsed (100 ps) laser focused on to a solid surface in vacuum. While it is intuitively obvious that $>10^{16}$ watt cm^{-2} laser intensity will create a hot plasma in the focal region, it is much less obvious that a toroidal magnetic field will be generated with a growth rate of 10^{16} G s^{-1}. Indeed it was not until 1971 that Stamper *et al.* (*Phys. Rev. Lett.* **26**, 1012; 1971) of the US Naval Research Laboratory (NRL) detected kilogauss magnetic fields with magnetic pick up coils 1 ~ cm from a laser-produced plasma, and gave a basic explanation of the field generation mechanism. Before this it had often been assumed that a desirable feature of laser-produced plasmas was the absence of magnetic field effects!

The pick up coils used by Stamper *et al.* measured the field only in the tenuous plasma resulting from free expansion into vacuum about $\sim 10^{-7}$ s after its creation and it was not possible to deduce the much larger field strengths occurring in the microscopic laser-produced plasma itself. The question was nevertheless of great interest because it seemed that the field strengths might be great enough to considerably modify the plasma behaviour with important implications for the developing ideas of laser fusion.

It was the NRL workers, Stamper and Ripin (*Phys. Rev. Lett.* **34**, 138; 1975), who tackled this problem by looking for, and observing in 1975, the rotation in the plasma of the plane of polarisation of a probe pulse of laser light by the magnetic field induced Faraday effect. Their results, indicating megagauss fields, were of a preliminary nature and subsequent efforts to reproduce them both at NRL and elsewhere were negative.

There developed a situation in which theoretical models of increasing sophistication such as that of Colombant and Windsor (*Phys. Rev. Lett.* **38**, 697; 1977) predicted the megagauss fields but only the doubtful NRL experiment had shown any direct evidence for the fields, and in particular very careful 4th harmonic optical probing experiments by Attwood at the US Lawrence Livermore Laboratory had shown no fields above 100 kG to be present when ~ 50 μm spherical glass shell targets were irradiated.

The paradox was resolved in 1978 when the NRL group devised a novel way to avoid a persistent problem of the optical probing experiments. 2nd or 4th harmonic generation using a fraction of the main laser pulse had hitherto been used as a convenient source of a synchronised optical probe pulse but the measurements were complicated by the emission of those same harmonics from the plasma itself. The solution found was to generate the 0.53 micron second harmonic but to further shift its wavelength by stimulated Raman scattering in ethanol to 0.6329 micron. This eliminated interference from plasma generated harmonics and in May 1978 Stamper *et al.* were able to report irrefutable confirmation of their earlier experiment showing megagauss fields (*Phys. Rev. Lett.* **40**, 1177; 1978) but there remained an apparent conflict with the absence of such fields in the microballoon target experiments of Attwood.

It was perhaps an illustration of the coming of age of the new SRC Central Laser Facility in England that Raven, Willi and Rumsby, having an experiment scheduled just a few weeks later than that at NRL were able to take advantage of the Raman shifting idea and further to improve the experiment by the addition of simultaneous interferometry. The latter enabled direct determination of the electron density distribution which must be known to unfold the magnetic field from the Faraday rotation angle θ since $\theta \propto \int_l B.n_e dl$. They produced an elegant set of results which gave the most accurate field determinations to date. Moreover their results removed the remaining discrepancy by confirming Attwood's low field values on 50 μm spherical targets and Stamper *et al.*'s megagauss fields on plane targets.

The current state of the art may be summarised by saying that the megagauss toroidal fields and associated tens of kiloampere currents do occur. The primary source of the field is known and in simple terms involves an imbalance in the diffusive flow of electrons in the plasma creating a current loop (Figure 1). The axial increase of electron number density N_e leads to an axial outflow of electrons and the radial decrease of T_e leads to a radial fall-off in this outflow. Charge conservation requires a current loop as illustrated and this thermoelectric type of generator creates a field increasing with time with

$$\frac{dB}{dt} \propto \nabla T_e \wedge \nabla n_e$$

and having a toroidal geometry as seen experimentally. The magnitude of the field is such that it has a significant pressure relative to the plasma (45% in the data of Raven *et al.*) and has a strong effect on electron diffusion by creating an electron cyclotron orbit diameter considerably smaller than the field free 90° deflection mean free path (7% in the data of Raven *et al.*).

In conclusion it is of interest to consider areas requiring further study. The relative importance of several other processes producing the magnetic field in addition to $\nabla T_e \wedge \nabla n_e$ is not yet fully clear. The effect of the field on heat flow

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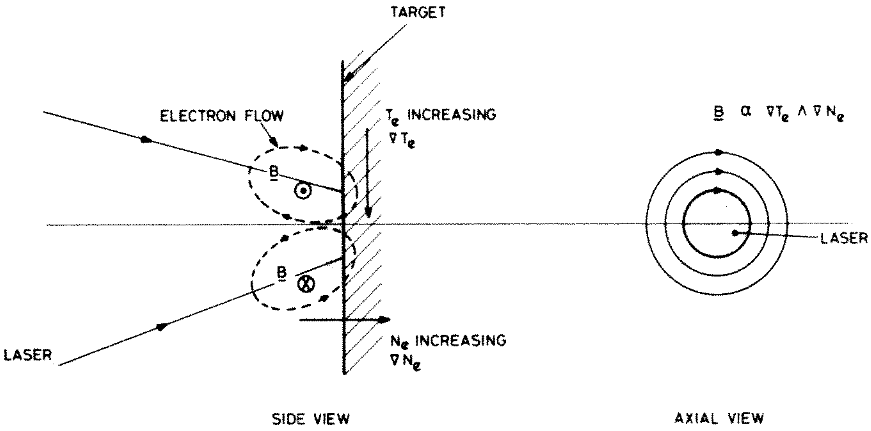

Fig. 1 Schematic diagram showing the target laser beam current path and magnetic field.

is also uncertain, first at the theoretical level as discussed by Max *et al.* (*Phys. Fluids* **21,** 128; 1978) and second because no experimental data have been obtained on the field strengths in the region of a plasma of above critical density where heat transport by electrons is the dominant process. ☐

Calcium control of enzyme reactions

from Michael J. Geisow

THE diverse roles attributed to calcium ion as a mediator of biological control have proliferated to such an extent, that the symbolic representation of this element at the focus of schematic cells has become a common sight in reviews. Many intracellular calcium-binding structures, membrane complexes as well as discrete proteins, have been reported. Which of these can be considered as sites of calcium-mediated control? The normal ionised calcium level in the cytosol of cells is low (0.1 to 0.01 μM). Even under conditions of 'activation' like the response to a hormone, calcium ion concentration probably does not rise much above 10 μM. Moreover, this increased concentration is not uniform because of diffusional barriers in the cytosol, and it is transient because of the action of membrane calcium pumps.

Subcellular structures which are potential targets of calcium dependent regulation, must consequently have dissociation constants for calcium of the order of 1 μM. Additionally, since intracellular magnesium concentration is relatively constant at 1 mM, calcium binding sites must have a selectivity of at least 10^3 for calcium over magnesium. As far as is known, only proteins can meet these calcium-binding requirements.

The most universally accepted protein effector of calcium-mediated control is troponin C (TnC), which makes muscle actomyosin ATPase sensitive to free calcium above about 1 μM (see *News and Views* **275,** 177; 1978). TnC has four divalent cation binding sites, of which two can only bind calcium. The protein molecule responds to bound cations with a large conformational change. TnC could be called the archetypal calcium receptor protein, since once actomyosin was recognised in non-muscle cells, the presence of proteins analogous in function to TnC was also anticipated. Kuo and Coffee (*J. biol. Chem.* **251,** 1603; 1976) isolated just such a protein from adrenal glands. Physically and chemically similar to TnC, this molecule bound two calcium ions with an apparent dissociation constant of 20 μM. This resulted in a considerable conformational transition, analogous to that undergone by TnC. Although it seems probable that this protein could activate actomyosin, the actual process regulated by the adrenal TnC remains undetermined.

By contrast, a variety of functions have been attributed to another intracellular calcium-binding protein, first identified in brain. This molecule, known as the calcium-dependent regulator or modulator protein (MP) has been purified from a wide variety of tissues. Although MP has chemical and structural homology with TnC, it is quite distinct and is two orders of magnitude less effective in stimulating actomyosin ATPase. At 1 mM Mg^{2+}, calcium ions bind to MP with an apparent dissociation constant of 3 μM (Wolff *et al.* in *Calcium Binding Proteins and Calcium Function*, 97, North-Holland New York; 1977). The modulator protein activates cyclic nucleotide phosphodiesterase, adenylate cyclase and red blood cell Ca^{2+}-dependent ATPase (Parett & Penniston *J. biol. Chem.* **253,** 4676; 1978). Both MP and TnC inhibit and reverse microtubule assembly in the presence of 10 μM Ca^{2+} (Marcum *et al. Proc. natn. Acad. Sci. U.S.A.* **75,** 3771; 1978). Indeed MP was localised by immunofluorescent labelling in the microtubule-rich mitotic spindle apparatus, implying its participation in the control of mitosis.

Many workers have found that the phosphorylation of membrane proteins is calcium sensitive. For example, Kruger *et al.* (*J. biol. Chem.* **252,** 2764; 1977) demonstrated that depolarisation of intact nerve-endings stimulated specific membrane protein phosphorylation, provided that this stimulus was delivered in a calcium-containing medium. Similar covalent phosphate incorporation occurred during histamine release from most cells (Sieghart *et al. Nature* **275,** 329; 1978). Recently Schulman and Greengard (*Nature* **271,** 478; 1978) showed that the phosphorylation of isolated nerve-ending membrane occurred in response to calcium and soluble cytoplasmic 'factors'. Purified MP was able to substitute effectively for the cytoplasmic proteins in the phosphorylation reaction. A common feature of the apparently diverse functions of MP is its connection with phosphate ester breakdown or synthesis. The essential link between all of the observations may have been provided by the identification of MP as a subunit of two protein kinases. Cohen *et al.* (*FEBS Lett.* **92,** 287; 1978) and Barylko *et al.* (*FEBS Lett.* **90,** 301; 1978) report that MP is a subunit of muscle phosphorylase kinase and myosin light chain kinase respectively. These kinases are sensitive to 1 μM Ca^{2+}, and it is likely that regulation occurs through the interaction of the intrinsic MP with a regulatory subunit of the kinases.

The modulation of cyclic nucleotide phosphodiesterase, adenylate cyclase and membrane Ca^{2+}-stimulated ATPases also seems to occur as the result of reversible binding of a calcium-MP complex to a regulatory subunit of these enzymes. It is interesting to speculate whether the regulation of microtubule polymerisation might result from an interaction of MP with tubulin GTPases (see David-Pfeuty *et al. Nature* **272,** 282; 1978.)

Although the status of TnC and MP as calcium-dependent regulators is now established, the way in which non-muscle cells could become activated as

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a result of contraction or phosphorylation/dephosphorylation processes remains obscure. In secretory tissues the universal response to raised cytosolic calcium is the release of secretory products stored in subcellular vesicles by fusion of vesicle and plasma membrane. Creutz *et al.* (*J. biol. Chem.* **253,** 2858; 1978) report the isolation of a 47,000 molecular weight protein which promotes the close association of adrenalin-containing granules isolated from adrenal glands. Contacts resembling the apposition of secretory granule membrane with plasma membrane before fusion, form reversibly in the presence of calcium ions above about 10 μM. It was not established whether the granule-aggregating protein itself bound calcium, or became bound as a result of a calcium binding event on the granule itself. The latter alternative is more consistent with the observations of Gratzl *et al.* (*Biophys. biochim. Acta.* **470,** 45; 1977). These authors claim that aggregation and fusion of isolated secretory granules from several tissues occurs in the presence of 10 μM calcium. Magnesium ions could not substitute for calcium in this process. A protein, rather than a lipid calcium-binding component, is implicated, since liposomes made from the lipids found in secretory granules do not aggregate or fuse at such low calcium concentrations.

The ability of the TnC-like proteins to confer calcium sensitivity upon enzymic processes, perhaps by combining with a common regulatory protein subunit, potentially accounts for the multiple intracellular effects of calcium. However, it is still premature to claim the uniqueness of these proteins as calcium receptors in non-muscle cells, and the characterisation of other recently identified members of this class of calcium-binding proteins is eagerly awaited. ☐

The enigma of poxviruses

from Arie Zuckerman and Charles Rondle

THE global eradication of smallpox is probably the greatest public health achievement of the 20th century. Eradication was possible because of the availability of a highly effective vaccine prepared from vaccinia virus. Yet one of the most controversial aspects of vaccination is the origin of vaccinia virus, and in particular whether it is derived from smallpox or cowpox. It has also been suggested that vaccinia might have arisen as a hybrid between these viruses. It appears, however, that vaccinia virus is most closely related to cowpox. Phylogenetically, the whole group probably derives from one common ancestor and its differentiation must be connected with the domestication of animals by the first agricultural settlers of the human race.

The second reason for the success of the smallpox eradication programme is that smallpox is a human disease with no known animal reservoir or insect vector. But nevertheless, problems remain since natural poxvirus infection of nonhuman primates does occur (see **241,** 425; 1973).

Monkeypox virus was first identified in 1958 during an outbreak of a vesicular skin disease in captive *Cynomolgus* monkeys in Copenhagen (von Magnus *et al.*, *Acta Path. microbiol. scand.* **46,** 156; 1959). The distribution of the rash was similar to that of smallpox in man and the virus was recognised as a poxvirus by its structural features under the electron microscope and by its serological relationship to vaccinia virus. By 1969, monkeypox virus had been isolated from captive nonhuman primates, or from their organs, on 10 occasions, including one isolation from an anteater which temporarily shared a cage with an infected orang-utang. Between 1970 and the middle of 1978, human cases of monkeypox were reported from West and Central Africa with five (15%) deaths, a mortality rate similar to smallpox in that region. Furthermore, most cases clinically resembled smallpox. The prospect of the spread of monkeypox from man to man was alarming, but in the event possible secondary transmission only occurred in two cases—of 55 susceptible family contacts only 2 (3.6%) acquired the infection, and there have been only 33 recognised cases among over 200 million people in a period of more than 8 years. But the natural host and vector of monkeypox virus remain unknown, despite the fact that there are reports of 'smallpox' in monkeys as long ago as 1767 (Arita & Henderson *Bull. Wld Hlth Org.* **39,** 277; 1968).

Whitepox virus is a more worrying puzzle. This virus was first identified in a Dutch laboratory in September 1964 during routine processing of Asian *Cynomolgus* kidney tissues (Gispen & Brand-Saathof, *Bull. Wld Hlth Org.* **46,** 585; 1972) and could not be distinguished at the time from smallpox virus by laboratory tests. The name 'whitepox' was given to the isolates because in contrast to monkeypox they produced small dense white pocks on the chorioallantoic membrane of the developing chick embryo. Between 1971 and 1973, this virus was isolated on four occasions from primate kidney tissues derived from a chimpanzee and a sala monkey, and from two rodents, *Mastomys natalensis* and a squirrel-like rodent captured in Zaire (Marennikova *et al. Bull. Wld Hlth Org.* **46,** 61; 1972; *Acta Virol.* **20,** 80; 1976). Experimentally, a whitepox virus inoculated into *Ceropithecus aethiops* monkey caused a generalised rash and illness. Whitepox virus still cannot be distinguished from smallpox virus by laboratory tests (Fenner *Prog. med. Virol.* **23,** 1; 1977), and it therefore remains both a puzzle and a threat. The fact that no infections with smallpox-like virus have occurred in Zaire during the 7 years since the last smallpox case was identified is at least reassuring. It does seem, therefore, that animal poxviruses are not a threat to the global eradication of smallpox, but the need for constant surveillance and laboratory studies continues.

Marennikova and Shelukhina (see this issue of *Nature,* page 291) now report that whitepox viruses isolated

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NATURE

A hundred years ago

THE *Daily News* Quebec correspondent telegraphs as follows:—"After long study and many experiments Prof. Bell has made an important discovery in connection with the telephone. It is well known that the telephone has been a comparative failure in England on account of the fatal induction generated by the contiguity of other wires. Prof. Bell has discovered simple and efficacious means whereby not only is induction prevented, but the clearness and force of the telephonic vocalisation greatly increased. Prof. Bell tells me that practical demonstration of the importance of the discovery will be given in London as soon as the necessary preliminaries are complete." We give this statement as published in the *Daily News,* though it must be received with some caution.

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from apparently healthy monkeys and rodents are stable clones which are present in populations of monkeypox virus. Inoculation of hamsters with monkeypox virus resulted in asymptomatic infection but virus was isolated from the hamsters for up to 7 weeks after inoculation. Four out of the 35 isolates showed that these were stable viruses identical with natural whitepox and which could not be differentiated from smallpox. It seems likely that the white clones were present in the original inoculum and were preferentially selected. This selection occurs in an unpredictable manner. Since whitepox viruses cannot be distinguished from smallpox virus, the results may be interpreted as an indication of the possible origin of smallpox viruses from monkeypox. □

What is climatology?

from T. M. L. Wigley and P. Brimblecombe

JUST what is the meaning of climatology as a discipline, and what qualifications or interests does one have to have in order to contribute to climatology? The recent conference 'Evolution of Planetary Atmospheres and Climatology of the Earth' in Nice* emphasised, perhaps more strongly than any similar gathering, the range of fields which now contribute to our understanding of past climates. Very few of the participants could call themselves climatologists in the traditional sense of the word; they came variously from astronomy, geology, space physics, geochemistry, statistics, glaciology, oceanography, botany/palynology and other areas of science.

The conference itself had very ambitious aims There were papers dealing with climatic change on all time-scales, from about 10^9 years down to seasonal changes, beginning with a review of the longest time-scale changes on Earth, Mars and Venus by T. Owen (State University of New York, Stony Brook). He pointed out some of the problems with the assumption that the Earth originally had a highly reducing atmosphere (NH_3, CH_4), and stated the present general view that CO_2 and H_2O were important original constituents. This hypothesis was strongly supported by A. Henderson-Sellers (University of Liverpool) and A. J Meadows (University of Leicester) who gave results from a model which, on the basis of a CO_2–H_2O atmosphere, was able to explain the so-called 'faint Sun paradox': that is, the apparently anomalous lack of extensive ice cover on the Earth during its early history when the solar output was substantially less than today. A number of other papers dealt with this contentious issue, an issue that has ramifications for the origin of life on Earth. Most experimental synthesis of the prebiotic formation of organic molecules begin with a strongly reducing atmosphere, but this and other recent work suggest that such a beginning is not a prerequisite for the evolution of life.

Bridging these and shorter time-scale studies was the study of mid-Cretaceous global climate presented by P. Larsson (University of Lancaster) which suggested that dinosaurs may well have been warm-blooded animals. On the 10^6 to 10^7-year time-scale, N. J. Shackleton (University of Cambridge) presented stimulating evidence on Tertiary climatic change which is at least as challenging to the theoretician as the faint Sun paradox. In the mid-Tertiary, for a period of $\sim 10^7$ years, it seems that tropical and subtropical climates were some 4 to 6 °C cooler than today: that is, colder than during any of the 17 or so glacial cycles which have occurred in the past 2.5×10^6 years. For the climatologist, such a climate picture is, at present, very hard to understand. It lies outside the possible realm of Milankovitch effects which are now generally accepted as being fundamental to the more recent glacial cycles. The Milankovitch theory was, however, treated with healthy scepticism in W. Broecker's review paper (Columbia University). The paper by A. Berger (Louvain University, Belgium) indicated that monthly insolation data may be more important in determining the climate-orbit link than the data used by Broecker, but Broecker's suggestion that there may still be important internal mechanisms was reinforced by some interesting modelling experiments reported by M. Ghil (New York University) and K. Bhattacharya (Columbia University). Their work, though based on very simple models, produced purely internal cyclic variations with nearly square-wave behaviour. They impose an imaginative albedo-temperature relation to obtain this cyclic solution. The amplitude of the cycles is reasonably in accord with past evidence, a particularly interesting factor since the modelling experiments based on external forcing (*à la* Milankovitch) invariably fail to obtain a climate signal with realistic amplitude These models were represented by G. R. North (University of Missouri) and J. A. Coakley's (National Center for Atmospheric Research, Boulder) elegantly simple seasonal model, and by the models discussed by E. Bernard (Institut Royal Météorologique, Brussels) and by C. Nicolis (Institute d'Aéronomie Spatiale, Brussels) (although the latter's efforts were directed towards longer time-scale climatic fluctuations), and, in a later session by the work of M. Hantel (Bonn University).

The Milankovitch theory was once again strongly supported in the paper by J D. Hays and D. W. Cooke (Lamont-Doherty Geological Observatory, Columbia University). They showed that major climatic fluctuations were approximately in phase between Northern and Southern hemispheres over the past 5×10^5 yr, and that the well-established periods of 23,000, 41,000 and 100,000 yr also show up in Antarctic deep-sea cores. These authors also suggested that the glacial to interglacial transition, as evidenced by a reduction (by a factor of ~10) in Antarctic summer sea ice, might have occurred in as little as a few hundred years, and that such changes preceded major ice volume changes in the Northern hemisphere.

Interesting papers on 3×10^5 and 3×10^4-yr time-scales were presented by J. C. Duplessy (Centre des Faibles Radioactivites, Gif-sur-Yvette) and C. Lorius (Laboratoire de Glaciologie, Grenoble), but the latter part of the conference was devoted to a much shorter time-scale. A brief review by T. M. L. Wigley (University of East Anglia) pointed out that the European Medieval warm epoch (approximately AD 1000–1200) was probably not a global phenomenon, in contrast to the apparent ubiquity of the climax of the Little Ice Age (AD 1500–1700). This picture provided a backdrop to a number of detailed descriptions of climatic events during the past 1000 years, descriptions which were highlighted by the interesting dendroclimatological studies of F. H. Schweingruber, O. U. Bracker and E. Schar (Swiss Federal Institute of Forestry Research, Birmensdorf), A. Berger and co-workers (Louvain University), and F. Serre (Marseille University). Schweingruber's group are pioneers in using wood density as a proxy climate indicator (closely related to summer temperature), and these workers showed some intriguing evidence of teleconnections between North America and Europe on the 5-year time-scale.

One of the most immediately significant contributions came from C. E. Leith (National Center for Atmospheric Research, Boulder, Colorado) on the mechanisms of short period climatic fluctuations He gave a most lucid explanation of predictability, based largely on the work of Madden If, as

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is so in the present state of the 'art', weather is essentially unpredictable beyond about a 5-day period, then the statistics of observed climate variability imply, necessarily, that month to month climate changes are, for many parts of the world, largely unpredictable. Although this is an undisputable statement of present capability, there are some rays of hope sneaking over the horizon For instance, there is some indication that, if we knew more about the mechanisms of 'blocking', then there would be greatly improved predictability in these situations. Furthermore, as S. H. Schneider (Boulder, Colorado) pointed out, we may still be able to develop good selective prediction schemes (for extreme events, for example) even without improving day to day forecasting skills or our understanding of the meteorology of blocking.

Schneider himself presented one of the most interesting review papers, on the effects of man's activities on climate. Although man's effects, on the regional and global scale, are not yet distinguishable from 'natural' climatic fluctuations, the potential for more significant effects is high. Because of the immobility of society *en masse* we are, in most places, unable to adapt rapidly to changes in the climate of marginal regions (be these changes natural or man-made), so possible man-induced changes are of considerable concern. In particular, the magnitude of CO_2 effects before the middle of next century could be 1 or 2 °C overall at the surface, and much greater in polar regions. This would have serious consequences. This point was reinforced in an engrossing summary given by Professor H. Flohn (Bonn University). He considers two factors of prime future importance. First, in the past there is strong evidence of extremely rapid climatic change—as fast as, for example, a 5 °C drop in summer temperature in less than 100 years. Should this happen in the future, there could be dramatic consequences. Second, the production of CO_2 by fossil fuel burning is liable to be a major problem within 100 years. On a reassuring note, however, Flohn pointed out that even a large warming would probably not, as some people have suggested, cause a rapid melting of the Arctic sea ice. Partly because of feedback effects and partly due to the large time constant for the Greenland Ice Sheet the minimum time for such melting would be at least 1000 years And, although this is certainly not a cause for complacency, we are still not absolutely certain that increased CO_2 will lead to warming. All models used to date to examine this question have failed to handle possible cloudiness changes in a realistic way: we just do not know how to do so at present. Still, the various models, many of which parameterise clouds very differently, all predict warming, so the odds are strongly in favour of such an outcome and it is a possibility which must be taken with utmost seriousness.

Although many significant advances have been made in climatology in recent years, it is sobering to consider these in the light of the first climate model; that of the Egyptian scribe who said, around 3000 BC, "the Sun warms the Earth". ☐

Histone genes displayed

from P. J. Ford

HISTONE proteins are of central importance to the molecular biology of higher organisms since with DNA they are involved in chromatin structure and function. The five types of histone, H1, H2A, H2B, H3 and H4 are found combined with an equal amount by weight of DNA in nuclei of eukaryote cells. The relative amount of each histone type appears to be regulated since they are present in equal molar amounts, except H1 at half molar amount. In addition there are in many organisms developmental stage and tissue-specific sequence variants within each of the major histone groups. Quite how these variants relate to chromatin structure and function is not clear, but they provide a useful handle for studies on the control of specific gene expression during development and differentiation. As is often the case recent advances in understanding histone gene structure stem from the discovery of a particularly favourable system, early sea urchin development, in which rapid cell division demands considerable rates of histone synthesis to accommodate the high rates of DNA synthesis, and from which histone mRNA is readily extracted (Kedes & Gross *Nature* **223**, 135; 1969). Estimates of histone gene numbers in sea urchins suggested the presence of several hundred to a thousand copies of each histone gene depending on species (Kedes & Birnstiel *Nature new Biol.* **230**, 165; 1971). In this respect sea urchins may be an extreme case since other phylogenetically diverse groups have considerably fewer (20-100) copies of each histone gene.

Isolation by molecular cloning techniques of DNA segments coding for sea urchin histone genes has led to proof that genes for all five histone types occur together in one repeating unit in the order H4, H2B, H3, H2A and H1 and that each structural gene is separated from the next within the unit by spacer DNA. Recently (Schaffner *et al. Cell* **14**, 655; 1978) Birnstiel and his colleagues have presented DNA sequences for more than half the total histone gene repeat unit known as h22 from the sea urchin *Psammechinus miliaris*. These include complete sequences for the H3 and H2A genes plus almost complete sequences for the other three structural genes and entirely confirms earlier maps of this particular DNA fragment. From a comparison of known histone protein sequences with the amino acid sequences which would be generated by translation of these particular structural genes it seems likely that unit h22 is active in early development and does not code for the specific histone subtypes found in sea urchin sperm. Additional information derived from these sequences is that h22 histone coding sequences do not contain inserted untranslated sequences like those seen in mouse and rabbit globin and chicken ovalbumin genes. Histones are not synthesised as longer precursors and there is no evidence for substantial regions which might be translated in any other reading frame to give other types of polypeptide.

As well as sequences for structural genes Schaffner *et al.* have determined sequences for regions at the beginning and end, called prelude and trailing sequences, respectively, of the structural genes and for some of the spacer regions. As they state: "If it were true that identical functioning of the regulatory signals require exactly identical primary structures, then sequence comparisons between preludes and trailing sequences around different histone genes should reveal some sequence homologies Our sequence comparison by computer analysing runs of three or more nucleotides has been relatively unsuccessful in that it has disclosed no long runs of sequences held in common between different spacers or preludes, with the possible exception of a short sequence in two spacer regions and a sequence of twenty nucleotides in the prelude regions of the H2A and H4 structural genes". I suppose we are to conclude that either the sequences presented do not cover regulatory sites or that they function in a way which does not require extensive sequence identity.

Sequences and maps of restriction enzyme sites in spacer DNA of h22 has revealed that unlike the spacers found in *Xenopus* ribosomal and 5S RNA genes there are no small repetitious sequences present Consequently no spacer length variation like that in the

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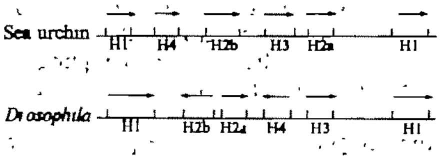
Xenopus rRNA genes due to the presence of different numbers of small repeating units is observed in sea urchin histone DNA. This is not to say that length variation does not occur, since Overton and Weinberg (*Cell* **14**, 247; 1978) studying independently isolated histone gene units from another sea urchin *Strongylocentrotus purpuratus* report that there is both length and sequence variation between different histone gene units. They have been able to show that this variation is found more extensively in histone DNA isolated from a group of sea urchins. More remarkable is that histone DNA variants from individual sea urchins are limited to a few of the major classes seen in the population as a whole. Each of five individuals were distinguishable on the basis of histone gene length and restriction site variants. Before attempting to assess what this means it will be necessary to follow histone gene patterns in offspring of individuals of known histone genotype.

During early sea urchin development two sets of histone genes are active. Kunkel and Weinberg (*Cell* **14**, 313; 1978) provide evidence that a full length histone DNA repeat, called pCO1, from *S. purpuratus* will hybridise to histone RNA from both cleavage and blastula stages. The hybrids formed with blastula transcripts are more easily melted (10 °C) and more easily digested with ribonuclease suggesting more extensive base mismatching than hybrids with cleavage stage transcripts and hence blastula transcripts probably derive from another type of histone gene. They suggest that pCO1 is preferentially transcribed during cleavage and not during blastula when transcription of other histone genes predominates. Such changes in transcription are also reflected by changes in histone protein synthesis (Newrock *et al. Cell* **14**, 327; 1978). Changes in the *in vivo* patterns of histone synthesis during early sea urchin development may be transferred to an *in vitro* protein synthesising system along with mRNA isolated from the appropriate developmental stages. There is no evidence from these studies for control of histone subtype synthesis at the translational level, since new subtype synthesis *in vivo* is closely coupled to appearance of mRNAs which can be translated *in vitro* to give products with very similar properties to the new histone subtypes seen *in vivo*.

Recently (Lifton *et al. Cold Spring Harbor Symp. quant. Biol.* **42**, 1047; 1977) the structure of *Drosophila melanogaster* histone genes has been reported. There are some interesting differences as well as similarities in comparison with sea urchin genes. Both contain all five histone coding sequences together in a single repeating unit, but the order and the direction of transcription differs. As the promoters for transcription have not been identified yet it is not clear whether each structural gene has its own promoter or whether there is a single promoter for each repeat unit, the resulting polycistronic primary transcripts being processed to give separate mRNAs. In *Drosophila* the fact that transcription must proceed in both directions makes a single promoter for both strands very unlikely. Lifton *et al.* have made an unsuccessful search for high molecular weight RNA molecules which hybridise to histone DNA and that might be considered polycistronic transcripts. They consider it most likely that *Drosophila* histone genes are transcribed independently, possibly from two divergent promoters and a single promoter. In sea urchins it is still unclear where transcription is initiated. The fact that clustering of different histone structural genes together in one unit is a very old and evolutionarily stable organisation suggests that it might be important perhaps in allowing the coordinated transcription of equal numbers of genes, thus ensuring correct histone protein stoichiometry, providing all histone messages are translated equally efficiently. □



A threat from fluorocarbons?

from a Correspondent

Do chlorofluorocarbons (CFCs) represent a threat to the ozone layer, with possible implications for life on this planet? This question, prompted by the hypothesis of Molina and Rowland (*Nature* **245**, 810; 1974), was addressed at 'Fluostrat '78'*. According to Molina and Rowland the breakdown in the stratosphere of CFCs, whose industrial production mushroomed in the early 1970s, would produce Cl and ClO which would reduce ozone through a catalytic cycle.

The representatives of the chemical industry went to great pains to point out the very large amount of work on this problem which has been sponsored by the Manufacturing Chemists Association. They pointed to the range of qualities which has made the CFCs such ideal propellants The search for alternatives has, so far, produced no compounds with even comparable properties. However, the case to be answered remains whether or not CFCs could reduce the amount of ozone in the atmosphere, thus allowing increased penetration of potentially harmful ultraviolet radiation.

The scientific proceedings of the symposium suggest that we are still some way from a definite answer to this question. Indeed, the interesting feature to emerge was the increasing awareness of the uncertainties in all the areas of research and of the great complexity of the system which atmospheric scientists are attempting to study.

A particularly important area of research is the investigation of the levels of CFCs present in the troposphere, in an attempt to determine their lifetimes there. J. E. Lovelock dealt with the difficulties in calibrating the electron capture gas chromatographs used in the measurement of CFCs. In a very elegant process, he has used an exponential dilution chamber to dilute accurately weighed amounts of CFCs to the parts per trillion (10^{-12}) level and then tested the calibration assumptions made by most experimenters. These assumptions have been found by Lovelock to be invalid over the range of mixing ratios measured in the atmosphere. Indeed, an intercomparison of a prepared standard sample of CF_2Cl_2 by 12 different laboratories showed very large differences between their findings.

It is apparent that the natural cycle of chlorine compounds in the atmosphere is not well understood. There was, in particular, much energetic debate regarding the apparently high levels of stratospheric ClO which have been reported by Anderson *et al.* (*Report to Congress and to the Administration of the Environmental Protection Agency on the National Aeronautics and Space Administration, 1978*).

B. A. Thrush of Cambridge University, in reviewing atmospheric chemistry, dealt with the importance of the coupling between the cycles of the nitrogen and chlorine compounds The theme of the importance of meteorological coupling mechanisms was taken further by R. J. Murgatroyd (Meteorological Office, Bracknell) and J. A. Pyle (Oxford University). The numerical models which have been used in predictive studies have generally been rather simple, one-dimensional (height only) representations of the atmosphere The need for models which represent meteorological processes rather better is apparent and these

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*Held in Brighton, UK on 5-6 October, and organised by the Society of Chemical Industry.

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models should include the important coupling between chemistry, radiation and dynamics. Model calculations of ozone depletion were shown to be sensitive to these feedback mechanisms.

The problem of just what effect an increase in ultraviolet radiation might have on the population was discussed by R. H. Mole of the Medical Research Council Radiobiology Unit, Harwell. He found faults with a wholly uncritical acceptance of the correlation between latitude and occurrence of skin cancer Changing social conventions in dress and increasing mobility of the population are certainly likely to be very important factors

In summary, the symposium in reviewing recent work highlighted many uncertainties There is clearly a need for a better understanding of the chlorine budget, the continuing measurement programme being very important. Further improvements in numerical models are necessary, with greater emphasis on the meteorology of the problem Finally, it would be of great help to the planners who must make some decision on the use of CFCs if a more reliable understanding of the possible medical effects of an increase in ultraviolet radiation dosages were gained. □

Microscopic optical potentials

from P. E. Hodgson

An international workshop on microscopic nuclear optical potentials was held at the University of Hamburg from 25 to 27 September.* Organised by Professor H V. von Geramb, it attracted over 100 participants and provided a welcome opportunity to review the recent progress in calculating optical potentials from the nucleon-nucleon interaction. Powerful methods of calculating the optical potential for finite nuclei have been developed by Jeukenne, Lejeune and Mahaux in Liège and by Rook and Brieva in Oxford (*Nature* **253**, 163; 1975 and **269**, 750; 1977). The resulting potentials fit the data quite well, without further parameter adjustment. This opens the way to a detailed understanding of the interaction of nucleons and groups of nucleons with nuclei, assists the phenomenological analyses of experimental data, and should stimulate further accurate experimental work The latest developments in these calculations were reviewed by C. Mahaux and by F A. Brieva, and related work by I. McCarthy (Adelaide) and M Weigel (Munich).

At lower energies, the structure of the target nucleus affects the interaction, and the effects of the coupling to the single-particle and collective states may be included in the calculation of the imaginary part of the potential by explicit summation over intermediate states. Work of this type was described by V. A Madsen (Oregon), N. Vinh Mau (Orsay) and Van Giai (Orsay), and applied to nucleon and alpha-particle scattering from ^{40}Ca and ^{208}Pb

It is now clear that the Saxon-Woods form factor universally used in optical model calculations is not always a sufficiently accurate representation of the potential. Improvements can be made by using microscopic form factors, or by supplementing the potential by an extra term depending on the orbital angular momentum L, or by altering the shape of the potential in a systematic way to fit the experimental data. A microscopic basis for an improved potential was provided by Y. C. Tang (Minnesota) who used the resonating group formalism to show that antisymmetrising the wavefunctions in a folding model calculation gives rise to parity-dependent terms in the potential. These take account of exchange processes and become less important as the mass difference between the interacting particles increases.

In some interactions there is a significant difference between the optical model predictions and the experimental data, and there is an obvious physical reason. It is then possible either to incorporate the physical effect in the calculations, or to add an extra phenomenological term to the potential. An example of this is the strong coupling to low-lying collective states which in the case of sub-Coulomb scattering of heavy ions causes the cross section to depart from the usual Fresnel form. This was discussed by A J. Baltz (Brookhaven), who showed that the data can be fitted by the addition of a long-range dynamic absorption potential that can be obtained in closed form Further analysis of this process was presented by G. Baur (Jülich). Another example is provided by the strong coupling to particular reaction channels This alters the elastic cross section in a way that can be fitted by an L-dependent potential, as shown by Mackintosh (Daresbury) He also showed the value of model-independent analyses of elastic scattering

Extensive work on the unification of the bound state and scattering potentials was presented by M M. Giannini (Genova), and other global phenomenological analyses by A. Tarrats (Saclay) and by H. Leeb (Vienna). A new method of measuring cross section ratios was described by S. M Austin (Michigan) and extensive measurements of proton scattering by light nuclei by M Pignanelli (Milano).

The analysis of the scattering of composite particles by nuclei has not yet been developed to the same stage of sophistication as nucleon scattering. Several folding models give semi-phenomenological potentials that often fit the data quite well, but much of the important physics, especially the effects of antisymmetrisation and exchange, is still hidden in the adjustable normalisation parameter A pioneer study of deuteron interactions was presented by A. A. Ionnides (Surrey) but apart from this the development of microscopic potentials for composite particles is still in the future. Meanwhile, a wealth of accurate data on the elastic scattering of deuterons and alpha-particles was presented by several groups, and in most cases they are very well fitted by optical model analyses

Calculations of the imaginary part of the optical potential for heavy ions were reviewed by D M. Brink (Oxford) who also described a new approach using the Feynmann path integral method. This can be used to relate the imaginary potential to the friction coefficient in heavy-ion scattering. D. F. Jackson (Surrey) showed how the extreme sensitivity of the alpha-decay rate to the potential barrier can be used to define the optical potential more precisely. A repulsive-core alpha-particle potential that gives the energies and widths of unbound cluster states and also fits the elastic scattering was described by K. A. Gridnev (Leningrad) Molecular states in heavy ion potentials were reviewed by W Scheid (Giessen).

A fully microscopic description of heavy-ion reactions is not yet practicable, but considerable progress has been made using the time-dependent Hartree-Fock method P G. Reinhard (Mainz) has used this method to determine the collective Hamiltonian and hence the quantum corrections to the optical potential

The meeting showed the extent and vitality of studies of all aspects of optical model potentials. The techniques for calculating microscopic potentials have now been established for nucleons, and much work remains to be done to improve the calculation of small terms and to apply the method to a wide range of nuclei and energies. At the same time it will be extended to composite particles, to link up with the very extensive phenomenological analyses that have already been made.

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*A summary of the workshop is available on request from P. E Hodgson. The papers will be published by Springer Verlag as a volume in the Lecture Notes in Physics series.

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review article

Advanced batteries

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Advanced-battery technology has been the basis of an Anglo-Danish interdisciplinary scientific project. The technological and economic aspects of advanced battery installations in vehicular and stationary applications are discussed in this article.

ADVANCED batteries provide a particularly good example of interdisciplinary science and technology. To gain the scientific understanding necessary to predict appropriate materials for constructing such devices and then technically to build a successful device, one must bring together such subjects as structural chemistry, solid- and liquid-state electrochemistry, interfacial and surface science, ceramics, corrosion science and metallurgy. Solid state physics and computer modelling of ionic motions within phases and across interfaces also have an important part to play. To assess the economic viability of particular systems one has to take account of projected battery performance parameters as well as such features as cost and availability of raw materials, processing technology, construction and fabrication costs, installation costs, safety, pollution and a comparison of these aspects weighed against alternative means of doing the same job.

An advanced battery project has recently started at several laboratories in the UK and Denmark. Seven institutions, four in England and three in Denmark, are collaborating to do research on materials for advanced batteries, together with technological and economic assessments of advanced battery installations in vehicular and stationary applications. The work is partially sponsored by the Commission of the European Communities.

β Alumina and the sodium–sulphur battery

Advanced batteries have stimulated much interesting work in solid-state chemistry and electrochemistry in recent years[1–3]. The seminal discovery was the observation of very high sodium ion mobility above room temperature in sodium β alumina by Weber and Kummer (at Ford) just over 10 yr ago[4]. β alumina, one of a family of closely related compounds, has a layer structure as shown in Fig. 1. Four layers of close-packed oxygen atoms contain four fold and six fold coordinated aluminium in an atomic arrangement analogous to that found in spinel, $MgAl_2O_4$. The formula of β alumina, however, $Na_{1+x}Al_{11}O_{17+\frac{x}{2}}$ is not compatible with an infinitely extending spinel structure. The relative lack of aluminium is reflected in the fact that every fifth layer of oxygen atoms is only one-quarter filled. The sodium ions are found in this relatively empty layer[5].

β Alumina was so named because it was thought to be an isomorph of aluminium oxide, and the crystal structure was essentially solved before the war[6]. However, the unusual electrical properties of sodium β alumina were not discovered until recently. When the electrical conductivity at 300 °C was measured, and it was realised that the current carriers were exclusively sodium ions and not electrons, the application of materials like β alumina to a modern generation of power batteries was quickly realised. In the sodium–sulphur battery, patented by Ford, instead of solid electrodes separated by a liquid electrolyte, as in the conventional lead–acid car battery, for example, sodium β alumina is used as a solid electrolyte, specifically conducting sodium ions, between liquid electrodes of sodium metal and sulphur (Fig. 2). The cell voltage, 2.08 V, is derived from the chemical reaction between sodium and sulphur to produce sodium polysulphide and the theoretical energy density, about 750 Wh kg^{-1}, is high compared with that of the lead–acid battery, only about 170 Wh kg^{-1}. Such impressive energy densities immediately indicated attractive possibilities for electric vehicle propulsion and other applications.

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The novelty of the properties discovered in sodium β alumina may be estimated from the fact that, although its melting point is around 2,000 °C, several A cm^{-2} of current may be passed across the electrolyte at 300 °C. The key to the rapid sodium ion mobility lies in the crystal structure and the low potential energy path for sodium migration this produces. β Alumina thus provides a happy hunting ground for molecular dynamics and potential energy calculations[7,8] as well as for experimental assessment of the effect of chemical structure variations and ion exchange on the electrical behaviour[9,10]. Many other cations including the other alkali metals and ammonium, protons and hydronium ions can be ion-exchanged for sodium by immersion in suitable molten salts or by other techniques[11].

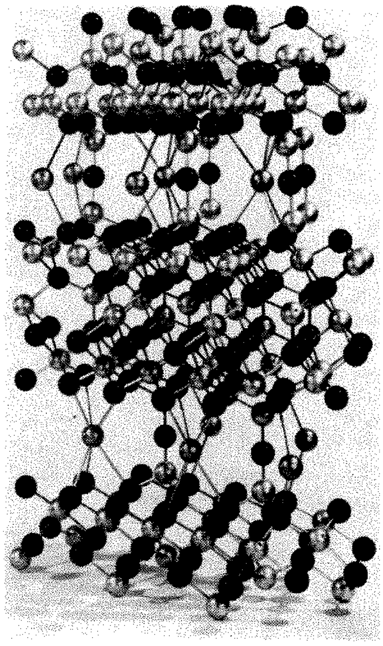

Fig. 1 The β alumina structure. The densely populated close-packed 'spinel blocks' containing aluminium and oxygen ions separate the mirror planes which contain the mobile sodium ions. The density of oxygen atoms in the mirror planes is only one-quarter of that in the spinel blocks. The separation between mirror planes is just over 11 Å.

Although the gross features of the β alumina structure have been known for a long time, the detailed crystallography of the mirror plane, vital to an understanding of the electrical properties, is still only partially understood. Recent work using neutron diffraction at Brookhaven National Laboratory has shown[12] that the occupation of the mirror plane is considerably more complicated than originally thought. Sodium β alumina as normally produced is invariably non-stoichiometric with x in the formula usually between 0.2 and 0.3, and it is now generally accepted that this non-stoichiometry is accommodated by extra oxygen as well as extra sodium occupying positions in the conducting plane. The actual conduction mechanism of sodium is, therefore, complex and has not yet been fully unravelled. Indeed, the mirror plane chemistry of the β alumina structure can almost be called a branch of science in its own right.

One interesting new development at Harwell has been the characterisation of hydrogen-conducting β aluminas. These have been studied electrochemically and gravimetrically[13,14] and by neutron diffraction[15]. A proton-conducting ceramic could have interesting applications, for example, as a medium temperature fuel-cell electrolyte.

An added complication in β alumina chemistry is that several variants of β alumina exist[9] and so-called β″ alumina, which has cubic rather than hexagonal stacking of the spinel blocks (as in β alumina) has a higher conductivity than β alumina but is more susceptible to attack by moisture and is not so rugged. A compromise between conductivity and strength may be obtained by having both β and β″ present in electrolyte tubes. High resolution electron microscopy has shown[16] that the mixing can occur on the unit cell level as well as by a mixing of macroscopic grains. The intergrowth of β- and β″-alumina layers is shown in Fig. 3. The higher conductivity of β″ alumina is related to the fact that the different stacking of the spinel blocks produces two equivalent cation sites in the mirror plane rather than one favoured site and one relatively blocking site as in β alumina, and also to the absence of interstitial oxygen in the mirror plane[12]. β″ alumina is non-stoichiometric with regard to sodium, an essential feature for rapid ionic motion, but this is compensated by magnesium substitution, $Na_{1+x}Al_{11-x}Mg_xO_{17}$ (with x usually between 0.2 and 1.0) or by lithium substitution rather than by interstitial oxygen.

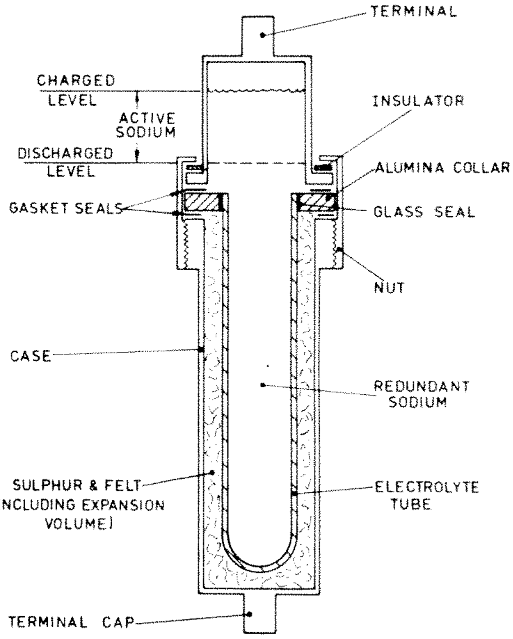

Fig. 2 A schematic model of the sodium–sulphur battery which uses a sodium β alumina solid electrolyte as the separator between liquid electrodes (sodium anode and sulphur cathode). The operating temperature is 300–400 °C.

The new science of rapid ion transport

The discovery of β alumina and the promise of the sodium–sulphur battery have had parallel effects in science and technology. Many programmes in several countries were set in motion to develop sodium–sulphur batteries[17]. Nevertheless, although development has been going on for 10 yr, batteries are not expected to be commercially available until the mid 1980s.

At the same time as the work on battery development, many scientific centres, particularly in the US and Europe, started programmes to try to discover new fast ion conducting solids, both for sodium, other alkali ions, and anions such as fluoride. For several years this search was not very successful, but as the structural requirements have become better understood alternative materials have begun to emerge (see, for example, refs

18–20). However, no sodium or other alkali ion-conducting materials have yet been found which show significantly improved electrical properties over β alumina and none has as low an activation energy for ionic mobility (0.13 eV) (ref. 21). Fast ion-conducting materials have been listed and discussed[22,23], and the field of solid state ionics and the technical applications, including advanced batteries, has recently been surveyed[30].

One new and important concept which has emerged, involving fast ion conductivity, is the insertion compound or solid-solution electrode[24]. In a solid-solution electrode there is both rapid ion transport and electronic conductivity. But a third vital ingredient is the presence of a range of stoichiometry involving the mobile ion. Fast-ion conducting materials of this type, therefore, can be used, not as electrolytes as is the case for β alumina, but as battery electrodes with either a liquid electrolyte or a solid electrolyte. In the latter case one is using fast ion-conducting materials to make an all-solid-state battery. In the former case, although the arrangement of solid and liquid phases is formally similar to that found in conventional lead–acid or alkaline batteries, the power-to-weight ratios obtainable using modern materials are much higher than was previously thought possible.

In contrast to solid electrolytes, where sodium is generally found to be the most mobile of the alkali ions, most good solid-solution electrode materials so far discovered act best as conductors of lithium ions, although this distinction may not survive further research. One of the most attractive solid-solution electrodes so far reported[25] is based on titanium disulphide, TiS_2. This is a layer material with adjacent layers of sulphur atoms held together only by van der Waals bonding (Fig. 4). TiS_2 and other similar chalcogenides of the transition metals can absorb alkaline cations between the sulphur layers. There is a slight expansion of the crystal axis perpendicular to the layers but otherwise no change in the crystal structure. For lithium going into titanium disulphide there is a continuous region of non-stoichiometry from TiS_2 to $LiTiS_2$. Moreover, the partial free energy of intercalation is relatively constant across the whole range. Titanium disulphide is quite light, cheap and readily available and the lithium–titanium sulphide battery developed by Exxon[26], which has an organic electrolyte and works at room temperature, has a theoretical energy density of 480 Wh kg^{-1}. This is already quite close to that of the sodium–sulphur couple, and there is the advantage of room-temperature operation, the absence of liquid alkaline metals, and, possibly, considerably reduced problems in fabrication and corrosion.

Solid-solution electrodes therefore provide a field of complementary scientific interest to solid electrolytes and have at least equal potential application in battery systems. Apart from the presence of rapid ion transport, the extra keys to the utility of solid-solution electrodes lie in the interfacial aspects of ions being able to pass directly between the electrolyte phase and the electrode without any change in crystal structure of the electrode or the necessity to form a new compound by electrocrystallisation (compare the electrode reactions in lead–acid and alkaline batteries), and the ability of the electrode to absorb a sufficient quantity of the active ion to provide systems with very attractive energy densities. The use of metal chalcogenides as reversible electrodes has recently been reviewed[27].

A third, more traditional, concept being applied in advanced battery design is the application of fused-salt electrolytes. The use of fused salts, of course, implies elevated temperatures, but fused salt electrolytes can allow the drawing of higher current densities than is presently possible with solid electrolyte-based batteries. The most notable current development using a fused-salt electrolyte is the lithium–iron sulphide battery, developed at the Argonne National Laboratory[28]. This battery operates at around 400 °C and uses a fused salt eutectic electrolyte mixture of lithium chloride and potassium chloride. Using a lithium–aluminium anode, the theoretical energy density is 650 Wh kg^{-1}, similar to that of sodium–sulphur, and the expected actual energy densities of the two systems are comparable.

The need for advanced batteries

Battery development is, therefore, at an interesting stage of transition and, although estimates differ and there will obviously be wide variations from country to country, considerable applications for advanced batteries can be foreseen in the medium- and long-term future. Future electrical energy supply will almost certainly have an increasing contribution both from nuclear power and from alternative sources such as wind, waves and solar energy. Irrespective of the relative importance of these various sources, there will be an increasing need for energy storage capacity to match the demand to the supply characteristics. Although desirable, a radically improved standard of energy economy does not change such a need. There are several ways of achieving such matching but electrical storage by secondary batteries offers one attractive method over a short timescale.

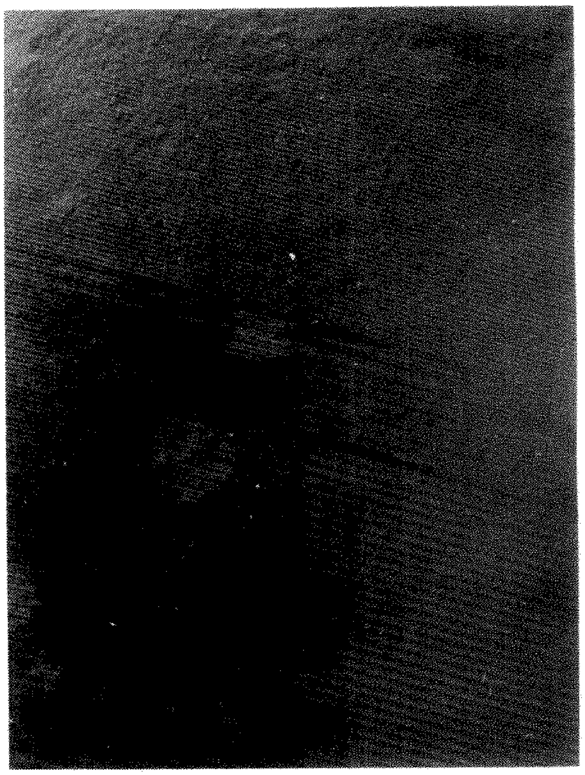

Fig. 3 A lattice image of a mixture of β alumina and β'' alumina showing intergrowth of the two structures at the unit cell level. The electron beam is parallel to the mirror plane. Although the spinel block thickness is the same in both materials, the space groups are different. Double diffraction is allowed in the $P6_3/mmc$ space group of β alumina and the 'forbidden' (001) reflections can be imaged in suitable conditions to generate 22 Å fringes. Only the space-group-allowed 11 Å spacing reflections are seen for β'' alumina.

Additionally, efficient off-peak energy utilisation by relatively non-polluting electric vehicles will surely play an increasingly important role in the total picture[29], although for such a role to be significant it is particularly important that electric vehicle technology is developed to provide products which are economically competitive with internal-combustion-engine-powered vehicles. The extent to which this can be achieved depends critically on the development of cheap, long-lifetime, high-energy batteries capable of many hundreds of cycles at deep discharge and high current density to provide the range and performance capabilities which will be demanded.

Advanced secondary batteries will therefore fulfil three roles in the field of efficient energy utilisation, or energy conservation in the medium- to long-term future: (1) load levelling in the electrical generating system; (2) smoothing of supply to demand for alternative energy sources; (3) electric vehicle traction; not only will this reduce dependence on oil supplies but will also improve urban air quality. Additional applications, of large industrial importance, such as nautical or aviation power systems, will, inevitably, also be found.

The second generation of advanced batteries

Rechargeable battery manufacture is still dominated by the lead–acid battery, but because of its low energy-to-weight ratio this system does not offer a very attractive solution to the roles just outlined. Industrial development of traditional alkaline electrolyte batteries (for example, nickel–zinc and nickel–iron) should produce improved power systems for vehicular traction in the relatively near future; thus the realisable energy density of a nickel–zinc battery is expected to be about 90 Wh kg^{-1}, over twice that of the lead–acid battery.

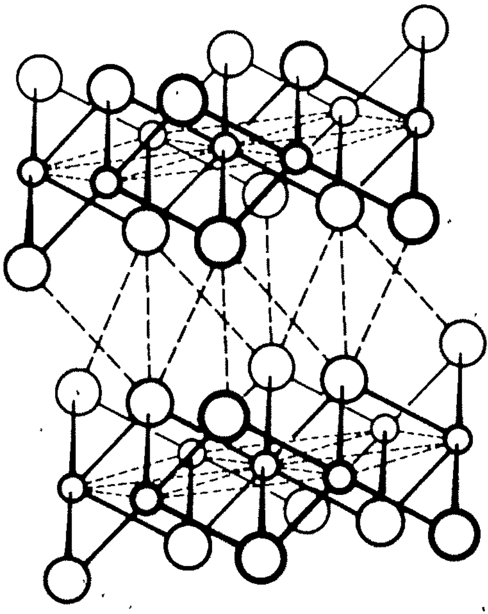

Fig. 4 The cadmium iodide structure adopted by titanium disulphide, TiS_2. The structure is layer-like with STiS sheets held together only by van der Waals interaction between the sulphur layers. The metal (titanium) atoms are in octahedral coordination by the anions (sulphur).

The present (first) generation of advanced batteries have considerably superior theoretical energy densities to the traditional types, but the weight penalty of engineering safe and reliable sodium–sulphur and lithium–sulphur batteries seems to be considerable, so that the realisable energy density may be no more than 150 Wh kg^{-1} for both these systems and perhaps may be as low as 110–120 Wh kg^{-1}. The improvement over the anticipated development of the traditional alkaline battery is not now so striking, especially when viewed against the need for high temperature operation, problems of reliability, safety, corrosion and so on. Moreover, it is estimated that the cost per kWh of these high temperature batteries will be similar to those of lead–acid and nickel–zinc; a desirable feature of an advanced battery would be reduced cost as well as improved performance.

Encouraging developments in basic scientific studies on solid-electrolyte synthesis and characterisation, and on solid–solution electrodes, do, however, promise that a new generation of advanced batteries may be possible without the disadvantages observed for sodium–sulphur and similar systems. Improved electrolytes, electrodes and electrochemical understanding of interfacial transport phenomena will, we hope, lead to cheaper, safer, and easier-to-construct second-generation advanced batteries.

The European basis for the new programme

Although much interesting work on new solid electrolytes has been undertaken in several scientific centres in Europe, there is little European industrial involvement so far with second generation ideas. Most of the interesting new developments and patents which might lead towards the second generation of advanced batteries have come from US industry. The example of the Li–TiS_2 battery has already been mentioned.

The Anglo–Danish programme aims to redress this imbalance. Although it will only run in the first instance for 18 months, to the middle of 1979, which is the end of the first 4-yr period of the European Commission's energy conservation programme, indications of success by this time may lead to a larger R & D role for advanced batteries in the next 4-yr programme. Industrial cooperation on promising developments will then be sought.

Although a European perspective for this programme, which is designed to lead to commercially exploitable products, involves a considerable re-orientation of attitude from the national perspective normally taken in such work, it is hoped that the wider view will lead to a better overall assessment of the commercial prospects for advanced battery systems.

The laboratories participating in the programme and the principal investigators, apart from the authors are: UKAEA Harwell Laboratory; Inorganic Chemistry Laboratory, Oxford University (Professor J. B. Goodenough); Department of Metallurgy and Materials Science, Imperial College London (Dr B. C. H. Steele); Department of Ceramics, Leeds University (Professor R. J. Brook); Odense University (Professor J. W. Perram); Danish Technical University, Lyngby (Dr K. Fl. Nielsen); Risø Laboratory (Dr O. Toft Sørensen).

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The Allende meteorite: evidence for a new cosmothermometer based on Ti^{3+}/Ti^{4+}

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Applications of the mineral thermodynamic condensation sequence to assemblages in Ca–Al–Ti-rich inclusions in the Allende meteorite suggest that the total amount of titanium and Ti^{3+} *contents of aluminous clinopyroxenes (fassaites) are temperature sensitive and that the ratio* Ti^{3+}/Ti^{4+} *may be used as a qualitative cosmothermometer.*

THE Allende meteorite was an observed fall in Pueblo Allende, Mexico in 1969 and it is classified as a type 3 carbonaceous chondrite. This meteorite has received unprecedented attention because it contains a suite of mineral inclusions which are in agreement with the predicted equilibrium thermodynamic calculations for phases condensing at 10^{-3} atm in the solar nebula[1–4], and because these inclusions probably represent some of the most primitive high temperature condensates yet encountered[5–8]. The sequences of mineral condensates, as a function of temperature, are summarised in Table 1 and although there is now uniform agreement that these inclusions, which are rich in calcium, aluminium and titanium, are relics of the primordial nebula, it is still unresolved from the evidence provided by chemical[9–17], mineralogical[18–24], and isotopic data[25–29], whether condensation ensued directly from a nebular gas to the solid[3,4], whether condensation gave rise to a liquid before solidification[18], or whether the inclusions are the refractory residue of chondritic composition remaining after intense heating and volatilisation[30,31].

Formation of inclusions

The differences of opinion on the formation of these inclusions, as primary condensates or as crystallisation products from a melt, depend partly on the interpretation of whether or not equilibrium conditions prevailed during the nucleation of mineral species in the early solar nebula. If the predicted solar thermodynamic mineral condensation sequence is followed (Table 1) we may assume equilibrium, and the calculations are based on an assumption of equilibrium[1–4]. However, if the inclusions are derived by crystallisation from metastable and supercooled condensed liquids, the kinetics of refractory solid–solid reactions argue against a continuation of equilibrium to lower temperatures[18]. Provided a distinction is made between inclusions that have high temperature affinities, and inclusions that have lower thermal stabilities, the question of equilibrium is largely reconciled. Only high temperature assemblages are considered here and the thermodynamic model of direct condensation to a solid is favoured.

The refractory inclusions in the Allende meteorite contain a variety of minerals with high titanium concentrations, and evidence is presented here which demonstrates that the valency states of titanium (Ti^{3+} and Ti^{4+}) vary as a function of temperature. Based on the distribution and the abundance of titanium, and on the systematics of mineral assemblages that are predicted from the thermodynamic calculations, it is proposed that Ti^{4+} and Ti^{3+} may be useful as a potential, but as yet uncalibrated, condensation cosmothermometer.

From the minerals listed in Table 1 and shown in Fig. 1, the major titanium phases in Ca–Al-rich inclusions in the Allende meteorite are: hibonite ($CaAl_{12}O_{19}$), 1–10 wt% TiO_2; perovskite ($CaTiO_3$), 58 wt% TiO_2; rhonite, $Ca_4(Ca, Mg, Al, Ti)_{12}(Al, Si)_{12}O_{40}$, 12–22 wt% TiO_2; and titanian–fassaite (clinopyroxenes in the quaternary system $CaMgSi_2O_6$–$CaAl_2SiO_6$–$CaTi^{4+}Al_2O_6$–$CaAlTi^{3+}SiO_6$), 2–18 wt% TiO_2. Calculations of

Fig. 1 *a*, The ranges of condensation temperatures for minerals condensing at 10^{-3} atm in the solar nebula; these data are listed in Table 1 and are from Grossman[3] and Blander and Fuchs[18] *b*, The same condensation sequence as a function of Ti^{3+} Titanium-bearing minerals are perovskite, fassaite, Ti_2O_3, anosovite, TiO_{2-x}, and rutile. Initial condensation temperatures for perovskite and for rutile are labelled limits denoting the upper and lower limits of Ti^{4+} stabilisation, the intermediate range in temperature is the region of Ti^{3+} and Ti^{4+} stabilisation. The dashed curve labelled TiO_{2-x} are Magneli phases or anionic deficient rutiles which may exist in the transitory interval between $Ti^{3+}+Ti^{4+}$ stabilisation and Ti^{4+} stabilisation exclusively. The location of Ti_2O_3 is considered, but dismissed as the peak stabilisation for Ti^{3+}, see text. The dashed and solid curves labelled fassaite are two alternative trends for Ti^{3+} as a function of condensation temperature. The dashed curve is implied because it would provide a symmetrical Ti^{3+} distribution; however, the solid curve is applicable as demonstrated by fassaite-bearing inclusions in Allende with Ti^{3+} decreasing as a function of decreasing *T* K (see Fig. 2)

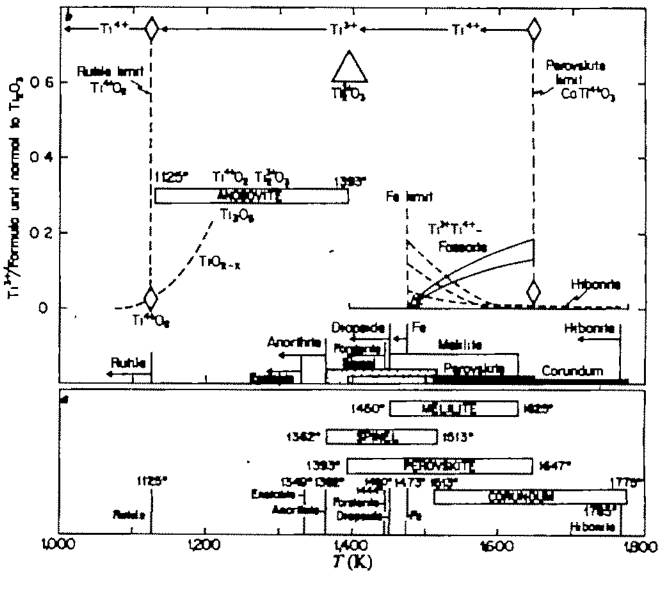

Table 1 The initial and final temperatures of condensation

Phase	Formula	Initial condensation temperature (K)	Temperature of disappearance (K)
Corundum	Al_2O_3	1,775	1,513*
Hibonite*	$CaAl_{12}O_{19}$	1,765	
Perovskite	$CaTiO_3$	1,647	1,393
Melilite	$Ca_2Al_2SiO_7$– $Ca_2MgSi_2O_7$	1,625	1,450
Spinel	$MgAl_2O_4$	1,513	1,362
Metallic iron	Fe(Fe–Ni)	1,473	
Diopside	$CaMgSi_2O_6$	1,450	
Forsterite	Mg_2SiO_4	1,444	
Anosovite	Ti_3O_5	1,393	1,125
Anorthite	$CaAl_2Si_2O_8$	1,362	
Enstatite	$MgSiO_3$	1,349	
Rutile	TiO_2	1,125	
Albite	$NaAlSi_3O_8$	~1,000	
Nepheline	$NaAlSiO_4$	~1,000	
Troilite	FeS	405	
Magnetite	Fe_3O_4	≤200	

Values taken from Grossman[3], except for * which are from Blander and Fuchs[18].

mineral composition data obtained from electron microprobe analyses, and based on the assumption of mineral stoichiometry, show that >95% of the titanium in perovskite is in the Ti^{4+} state, that ~10% of the Ti in hibonite is Ti^{3+}, that ~20% of the Ti in rhonite is Ti_2O_3, and that between 24 and 87% of the Ti in titanian–fassaite is also Ti^{3+}.

Thermodynamic considerations

Calculations for the predictive thermodynamic condensation sequence show that Ti^{4+} is stable in the mineral perovskite ($CaTiO_3$) at an initial temperature of 1,647 K; that Ti^{4+} and Ti^{3+} are stable in anosovite (Ti_3O_5) at an initial temperature of 1,393 K; and that Ti^{4+} is once again exclusively stable in rutile (TiO_2) at an initial condensation temperature of 1,125 K (refs 3, 4). This sequence of progressive stabilisation and destabilisation of Ti^{4+} is apparently, therefore, inconsistent with the widely held view that the condensing solar nebula becomes increasingly more oxidising with decreasing temperature. This is only an apparent anomaly because metallic titanium is stable at temperatures above perovskite. The interval between perovskite and rutile is in the interval of Ti^{3+} (and Ti^{4+}) stability, so that the presence of Ti^{3+} and the ratio of Ti^{3+}/Ti^{4+} should permit an estimate of the condensation temperature for the titanium-bearing minerals in that intermediate range which is constrained by the temperature limit of perovskite at the high end and by rutile at the lower end (Fig. 1).

To define the temperature range for the initial condensation of titanium-bearing minerals in the Allende meteorite the thermodynamic sequence must be rigorously followed and this sequence must be in agreement with the petrogenesis of the titanium-rich Ca–Al inclusions in Allende. Given these assumptions an analysis of the role of Ti^{3+} and Ti^{4+} in the condensation sequence now becomes possible. The absolute upper and lower limits of Ti^{4+} stabilisation are defined by perovskite (1,647 K) and by rutile (1,125 K), and values for intermediate Ti^{3+}/Ti^{4+} values are known for anosovite (Ti_3O_5 or $Ti^{4+}O_2$; $Ti_2^{3+}O_3$) to be in the range 1,393–1,125 K (refs 3, 4). Therefore, the peak stabilisation of Ti^{3+}, as $Ti_2^{3+}O_3$, ought to be above the upper limit of anosovite (1,393 K). Ti_2O_3 is therefore predictably stable between 1,647 K (perovskite) and 1,393 K (Fig. 1). If this peak stabilisation of Ti^{3+} is correct then the question arises as to whether the transformation of Ti^{4+} to Ti^{3+} is progressive on both the high temperature and low temperature sides of Ti_2O_3 (Fig. 1), or whether the transformation is abrupt. With respect to the low temperature side, the synthetic stable occurrences of non-stoichiometric rutile (TiO_{2-x}), known as Magneli phases[32–46] may be invoked, and the transition from anosovite to rutile may follow a smooth curve of progressively variable Ti^{3+}/Ti^{4+} concentrations at temperatures above 1,125 K (rutile) but below that of anosovite at 1,393 K (refs 47, 48). This proposed variation is shown in Fig. 1. The decrease towards Ti^{4+} stabilisation may also be applicable to the high temperature side of Ti_3O_5; however, an alternative is that the variation is simply a progressive decrease in Ti^{3+}, and in total titanium, in the range of condensation from perovskite to anosovite, that a peak in Ti_2O_3 is not attained and that both Ti^{3+} and Ti^{4+} are stable between 1,647 K (perovskite) and 1,125 K (rutile).

Discussion and results

To test these hypotheses the electron microprobe analyses of fassaites from the present study and from the literature[18–24,49] were recalculated assuming mineral stoichiometry, to determine the proportions of Ti^{3+} and Ti^{4+} in Allende pyroxenes. These

Fig. 2 Ti^{3+} plotted against total titanium for fassaites and associated pyroxenes and rhonite in inclusions 3898, All-4 and All-3,6. The data are derived from electron microprobe analyses with Ti^{3+} calculated, assuming mineral stoichiometry. ▲, Nos 1–15, and labelled *A–I* are from the literature listed in Table 2. ●, and the solid, slightly curved lines represent trends in zoned fassaites. Note that the rhonites are plotted on a reduced scale and in reality are at least a factor of two richer in Ti^{3+} than the associated fassaites. The horizontal tie-lines between rhonite and fassaite compositions are for rhonites that have decomposed to fassaite + perovskite + spinel. Open circles represent intra-crystalline fassaites. The dashed line marked ALL-4 is the overall trend for fassaite compositions (130 analyses) in this inclusion; these data are not plotted but are given in Haggerty and Merkel[47]. Arm, armalcolite, mineral 'T' to the new solid solution mineral; R_X, products of partial decomposition of mineral 'T' (ref. 53). Fassaites ions are based on 6 oxygens: rhonites are based on 40 oxygens (cation plot = 28/7).

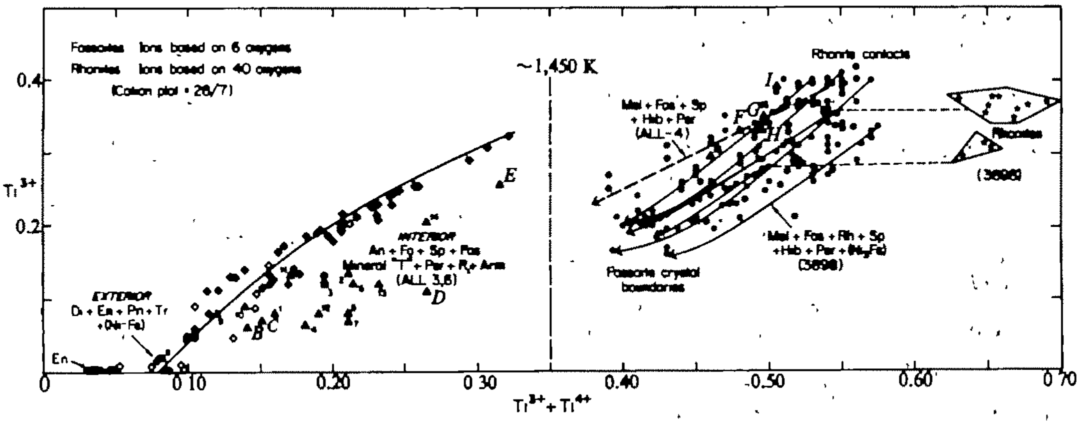

Table 2 Analyses of Ti^{3+}-bearing fassaites

	B	*C*	*D*	*E*	*F*	*G*	*H*	*I*	1	2	3	4
Mg	0.554	0.554	0.529	0.520	0.382	0.442	0.401	0.289	0.627	0.566	0.567	0.563
Al	0 877	0 806	0.814	0.745	0.871	0.782	0.823	0.989	0.646	0.697	0.719	0.764
Si	1.451	1.481	1.384	1.439	1.258	1.285	1.254	1.196	1.556	1.505	1.509	1.467
Ca	0.977	1.018	1.008	0.979	1.009	0.997	1.027	1.021	1.011	1.000	1.013	1.015
Ti^{3+}	0.060	0.070	0.112	0.254	0.344	0.338	0.324	0.390	0.080	0.128	0.122	0 062
Ti^{4+}	0.081	0.081	0 153	0.062	0.135	0.156	0.171	0.115	0.080	0 084	0.070	0.119
Fe										0.020		0.010
	4.000	4 000	4.000	4 000	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000
Cat. total*	3.97	3.97	3.93	3.92	3.90	3.89	3.89	3.88	3.96	3 96	3.95	3.98
Ti_2O_3	1.92	2.28	3.61	8.17	10.87	10.68	10.21	12.33	2.61	4 14	3.96	2.02
TiO_2	2.87	2.93	5.49	2.22	4.74	5.48	5.99	4.02	2,90	3.02	2.53	4.31
$Ti^{3+}/Ti^{3+}+Ti^{4+}$	0.426	0.464	0.423	0.804	0.718	0.684	0.654	0.772	0.500	0.604	0.635	0.343
	5	6	7	8	9	10	11	12	13	14	15	16
Mg	0.514	0.496	0.453	0.533	0.563	0.563	0.527	0.544	0.484	0 458	0.409	0.381
Al	0.836	0.861	0.947	0.884	0.794	0.774	0.810	0.796	0.869	0.865	0.890	0.907
Si	1.421	1.417	1.350	1 488	1.518	1.518	1.489	1.452	1.394	1.404	1 238	1 216
Ca	0 997	1.003	1.038	1.015	1.005	1.005	1.003	0.997	1.020	1 008	1.003	1.000
Ti^{3+}	0.080	0.122	0.070	0.020	0.080	0.090	0.132	0.080	0 120	0.204	0.288	0.342
Ti^{4+}	0.132	0.091	0.142	0.060	0.040	0.051	0.040	0.111	0.112	0.061	0.172	0 153
Fe	0.020	0.010						0.020				
	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4 000	4.000	4.000	4.000	4.000
Cat. total*	3.97	3.95	3.97	3.98	3 98	3 98	3.95	3.97	3.96	3.93	3.91	3 88
Ti_2O_3	2.59	3.96	2 26	0.66	2.62	2.94	4.29	2.59	3.88	6 58	9.21	10.80
TiO_2	4.75	3.28	5.10	2.19	1.46	1.85	1.45	4 02	4.02	2.19	6.11	5.37
$Ti^{3+}/Ti^{3+}+Ti^{4+}$	0.377	0.573	0.330	0.250	0.667	0.638	0.767	0.419	0.517	0.770	0.626	0.679

Analyses as listed by Mason[49], and are from the following sources: *B*, *E*, and *H* from Gray *et al.*[7]; *C* from Clarke *et al.*[9]; *D* from Marvin *et al.*[6]; *F* from Dowty and Clark[50]; *G* and *I* from Fuchs[20]. Analyses listed numerically (1–16) are from Grossman[22].

* Cationic totals assuming that Ti total is TiO_2. Any deviation to values <4.0 suggests Ti^{3+}. Recalculated TiO_2, Ti_2O_3 and normalised formulae assume stoichiometry.

results are presented in Tables 2 and 3 and Fig. 2. Although the confidence levels in these calculations are perhaps no greater than 95% it is relevant that independent optical spectra data[50–52] have demonstrated that Ti^{3+} is present in Allende pyroxenes and that the ratio of Ti^{3+}/Ti^{4+} in the fassaite studied is equal to 0.72 (Table 2 analysis *F*). Particularly close attention was given to monitoring the Ti standard during the microprobe analyses of the present study and repeated analyses were carried out so that the results are not only internally consistent, but are also consistent with data obtained by eight other laboratories. Although the bulk mineralogy of the inclusions from the literature cannot be directly related to those from this study there is uniform agreement among the data that Ti^{3+} is a major component of titanian–fassaites in the Allende meteorite. The proportion of Ti^{3+} correlates positively with total Ti content; and negative and positive correlations exist between Ti^{3+} and Al, and between Ti^{3+} and Si, respectively. These data, although broadly comparable among inclusions, are clearly related to a variety of inclusions which may, or may not, represent precisely identical thermal condensation intervals (Fig. 2).

Fassaite data from three coarse-grained Ca–Al–Ti-rich inclusions (0.5–1.0 mm in diameter) from Allende are shown in Fig. 2 as a function of Ti^{3+} against Ti^{3+}/Ti^{4+}. These inclusions, designated 3898, All-4 and All-3,6, contain the following assemblages: inclusion 3898 has rhonite as the earliest formed mineral. Rhonite forms cores which are mantled by titanian–fassaite and these rhonites are partially decomposed to spinel + perovskite + titanian–fassaite. The other minerals present are melilite + spinel + hibonite + perovskite. Minute blebs of Fe–Ni alloys occur along cracks in the inclusion in association with grossular garnet, and these minerals are considered to be secondary or reaction condensation products. Inclusion All-4 has the assemblage melilite + titanian–fassaite + spinel + hibonite + perovskite. Inclusion All-3,6 has two contrasting assemblages: an interior assemblage of anorthite + forsterite + spinel + titanian–fassaite + armalcolite + perovskite + a new mineral series along the solid solution join $R^{2+}Ti_3^{4+}O_7$–$R_2^{3+}Ti_2^{4+}O_7$ (where R^{2+} = Ca, Mg, Mn, Fe; and R^{3+} = Ti^{3+}, Cr, Al); and an exterior assemblage of diopside + enstatite + pentlandite + troilite + Ni–Fe alloys (Fig. 2).

The titanium contents of the fassaites from these three inclusions fall into two populations: one that is high in titanium and a second that is somewhat lower in titanium. The rhonite within fassaite crystals contains the highest total Ti concentration and the highest Ti_2O_3 value (20 wt% Ti_2O_3). The associated fassaites are compositionally zoned, contain 16 wt% Ti_2O_3 at rhonite contacts and a minimum of 6 wt% Ti_2O_3 at fassaite crystal margins. Fassaites in inclusion All-4 have an average Ti_2O_3 content of 9.94 wt% (range, 8.1–14.4 wt%), and an average TiO_2 content of 4.92 wt% TiO_2 (range, 2.04–6.73 wt%), obtained from 125 electron microprobe analyses. These data lie along a relatively smooth curve as summarised in Fig. 2 and are distinguished from the data for inclusion 3898 which occupy a broad band in the distribution of Ti^{3+} and total titanium. Fassaites in the interior of inclusion All-3,6 range from 7 to 10 wt% Ti_2O_3 whereas those on the margins of the inclusion decrease progressively to <1 wt% Ti_2O_3.

The condensation temperature for diopside ($CaMgSi_2O_6$), a member of the multicomponent pyroxene system, is calculated to be 1,450 K. It is reasonable, therefore, to assume initially that the more complex pyroxenes are within the range of this value, but that higher condensation temperatures are to be expected because of the extraordinarily high T and Al concentrations. Several temperature constraints for titanian–fassaites become apparent if the mineralogies of the Allende inclusions are integrated into the thermodynamic condensation temperature model (Table 1). These are as follows: (1) titanian–fassaite is paragenetically earlier than melilite ($Ca_2Al_2SiO_7$–$Ca_2MgSi_2O_7$), which has a condensation temperature in the range 1,625–1,450 K, this defines an upper limit of >1,625 K; (2) spinel ($MgAl_2O_4$) overlaps paragenetically with titanian–fassaite so that although the melilite upper limit remains fixed, the spinel (1,513–1,362 K) lower limit of 1,362 K now becomes effective; (3) titanian–fassaites are also present in association with forsterite (initial condensation $T = 1,444$ K) and anorthite (initial condensation $T = 1,362$ K), but not with enstatite (initial condensation $T = 1,349$ K), and this distribution now moves the lower limit from 1,362 K (spinel) to 1,349 K. Condensation temperatures for titanian–fassaites, therefore, are considered to be in the range >1,625 K–1,349 K.

Assuming this temperature range, and in association with the mineral chemistries of the three inclusions (3,898, All-4 and All-3,6), relative temperatures of initial condensation may be estimated. Because rhonite has re-equilibrated to titanian–fassaite+spinel+perovskite in 3898, and because rhonite has the largest concentration of titanium and the highest proportional content of Ti^{3+}, it is assumed to have condensed at $T > 1,625$ K, but $T < 1,647$ K (initial condensation T K of perovskite). Rhonite may have condensed before perovskite but that cannot be unequivocally demonstrated. Inclusion All-4 is inferred to have condensed in the interval $T = 1,450$–$1,625$ K, based on melilite stability; it may have condensed at temperatures slightly higher than 1,625 K but not as high as 1,647 K, or as high as the rhonite-bearing inclusion 3898. The lower limit of condensation for inclusions 3898 and All-4 is defined by the condensation temperature of iron at 1,473 K. Neither of these inclusions have either metallic iron or FeO in mineral analyses and the terminal condensation temperature must, therefore, have been higher than 1,473 K. Inclusion All-3,6 is clearly a lower temperature condensate based on the assemblage anorthite+forsterite+diopside+enstatite (Table 1, Fig. 1), the presence of Ni–Fe alloys, and the presence of FeO in spinel, armalcolite, olivine, the new mineral series ($R^{2+}Ti_3^{4+}O_7$–$R_2^{3+}Ti_2^{4+}O_7$), and titanian–fassaite[53]. The upper limit of condensation, therefore, is $T < 1,473$ K, the initial condensation temperature of metallic iron. The lower limit for this inclusion is defined by enstatite, at the margins of the inclusion, at $T < 1,349$ K, but reaction continued in the condensing nebula to temperatures as low as 405 K (troilite) before incorporation into the carbonaceous matrix.

Table 3 Electron microprobe analyses for fassaites in inclusions 3898 (1–4), ALL–4 (5–8) and ALL-3,6 (9–12)

	1	2	3	4	5	6	7	8	9	10	11	12
MgO	7.46	6.55	5.62	6.46	6.44	6.54	6.19	6.95	16.55	26.74	22.04	20.67
Al_2O_3	18.37	19.32	20.24	19.01	19.22	19.57	20.24	19.74	7.24	5.33	4.28	3.87
SiO_2	32.82	31.73	29.75	32.40	33.13	33.33	32.94	33.87	45.59	50.74	51.27	49.89
CaO	25.69	25.72	25.68	25.01	25.17	25.15	25 05	25.47	19.49	10.72	18.95	18.41
TiO_2	15.88	16.92	18.89	17.48	16.27	15.39	15.88	14.44	11.07	6.57	3 24	3.61
Cr_2O_3	0.1	0.07	0.07	0.08	0.01	0.02	0.01	0.01	0.53	0.44	0.26	0.32
MnO	0.06	0.08	0.08	0.01	0.05	0.03	0.04	0.02	0.01	0.06	0.08	0.22
FeO	0.01	0.04	0.03	0.01	0.03	0.04	0.04	0.04	0.09	0.31	0.34	2.45
	100 4	100.43	100 36	100.4	100.32	100.07	100.49	100.54	100.56	100.91	100.46	99.44
Mg	0.423	0.373	0 323	0.369	0.366	0.372	0.351	0.392	0.910	1.399	1.169	1.119
Al	0.823	0.870	0.919	0.858	0.866	0.881	0.908	0.880	0.315	0.220	0.18	0.166
Si	1.247	1.212	1.145	1.240	1.266	1.273	1.255	1.281	1.680	1.781	1.823	1.811
Ca	1 047	1.053	1.060	1.026	1.031	1.029	1.027	1.032	0.770	0.403	0.722	0.716
Ti^{3+}	0.229	0.267	0.306	0 348	0.324	0.302	0.316	0.256	0.304	0.140	0.005	0.00
Ti^{4+}	0.224	0.218	0.241	0.155	0.143	0.139	0.138	0.155	0.002	0.032	0.080	0.099
	4.000	4.000	4.000	4.000	4.000	4.000	4 000	4.000	4.000	4.000	4.000	4.000
Ti_2O_3	7.22	8.38	9.51	10.879	10.24	9.57	10.00	8.13	9.89	4.79	0.20	0.00
TiO_2	7.85	7.60	8.32	5.388	5.02	4.89	4.85	5.47	0.08	1 25	3.02	3.61
Av*	10	8	12	23	28	10	19	15	5	6	4	3

* Number of analyses averaged.

The complex but systematic decreases in zoned titanian–fassaites in inclusion 3898 (Fig. 2), the larger bulk titanium contents of fassaite at rhonite decomposition boundaries, and the relative displacements of fassaite titanium concentrations between inclusions characterised by melilite (3898 and All-4), and inclusions dominated by anorthite+forsterite (All-3,6), suggest that titanium dissolution in aluminium-bearing clinopyroxenes is temperature dependent and that these concentrations reflect the environment of solar nebula condensation. These data, therefore, show that total titanium and the proportion of Ti^{3+} decrease progressively with decreasing condensation temperature and that a peak in Ti^{3+} stabilisation, as the oxide Ti_2O_3, is not attained. The overall trends in Ti^{3+} distributions presented here may only be part of a more complex picture. Inclusions having melilite and anorthite, or inclusions in which the evolution of perovskite can be traced, are clearly required to provide information on the behaviour of Ti^{3+} at ~1,450 K (Fig. 2), and to establish whether the pyroxene trends are duplicated by other titanium bearing minerals.

Conclusions

I conclude that: (1) the calculated thermodynamic condensation mineral sequence is consistent with assemblages and compositions of phases in the high Ca–Al–Ti inclusions in the Allende meteorite; (2) because the predicted thermodynamic sequence is followed, the direct gas to solid condensation hypothesis is favoured; (3) based on these thermodynamic calculations the condensation interval of Ti^{3+} stabilisation is in the temperature range 1,647–1,125 K; (4) the Allende inclusion evidence and thermodynamic constraints suggest that titanian–fassaites condensed in the temperature interval defined by an upper limit of 1,625 K and a lower limit of 1,349 K; (5) the presence of Ti^{3+} in titanian–fassaites may be used as a qualitative cosmothermometer, with the ratio of $Ti^{3+}/Ti^{3+}+Ti^{4+}$ and total Ti content being high at high condensation temperatures and lower at correspondingly lower condenstion temperatures; and (6) rhonite-bearing inclusions have high initial condensation temperatures, which are followed by inclusions with titanian–fassaite and melilite, and these in turn are followed at lower temperatures by inclusions with anorthite+forsterite+diopside+enstatite, suggesting that the Allende meteorite has sampled a spectrum of the condensing solar nebula.

It is emphasised that the ranges of temperatures that are estimated for minerals in the Ca–Al–Ti rich inclusions in the Allende meteorite are subject to the limits of accuracy in the thermodynamic data. However, improvements to these data are unlikely to alter the overall conclusions, although the temperature estimates will undoubtedly be refined.

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Actinide activities in water entering the northern North Sea

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Surface seawater measurements of $^{239+240}$Pu, ^{238}Pu, ^{241}Am and ^{137}Cs have been carried out in the North Sea and western Atlantic. Statistical analysis demonstrated that the discharges by fuel reprocessing plants in the UK are affecting plutonium activities in these sea areas. A difference in behaviour of americium to plutonium was also observed.

THE North Sea is a multi-use coastal-sea area of great economic importance to European countries. As a development of the data collection on actinide element distribution in this region, a detailed investigation was undertaken in July–September 1976 by the FS Meteor of the northern North Sea and Western Atlantic, northwards of 56°30′ N till 65°00′ N bounded by 10°00′ W to about 8°00′ E. Fifty-eight sampling stations were measured for surface water activity of $^{239+240}$Pu and ^{238}Pu; ^{241}Am measurements were carried out at 16 stations. Caesium, strontium, ruthenium and tritium measurements were also undertaken and will be reported elsewhere (H.K. in preparation).

The number of results obtained in these sea areas permitted a statistical analysis of $^{239+240}$Pu and ^{137}Cs data for variations in distribution, this was carried out for three different sea regions An initial estimate has been made of the dispersion of plutonium and the identification of its source as being other than from fallout determined.

Methods

Collection and analysis of actinides and caesium have been described in detail elsewhere[1,2]. These methods have been used on IAEA intercalibration samples and the results obtained compared with other laboratories by Murray and Stathan[1]. Sampling techniques and statistical treatment for these intercalibration samples are discussed in detail by Fukai and Murray[3].

The present sampling procedure, which measures the total actinides in unfiltered samples, was carried out on-board the FS Meteor. A series of samples, from two stations, were analysed on-board and in Hamburg to demonstrate that the precipitation carried out at sea and subsequent handling had no systematic effect on the results. No differences in the water activities obtained by the two methods could be detected. Monitoring of the chemical yield solutions before and after the cruise showed that no changes had occurred in their activity during the 2-month period.

The reproducibility of the method for both plutonium and americium was checked by taking replicate sets of samples at three stations, those at 58°36′ N, 02°52′ W; 58°30′ N, 05°29′ W

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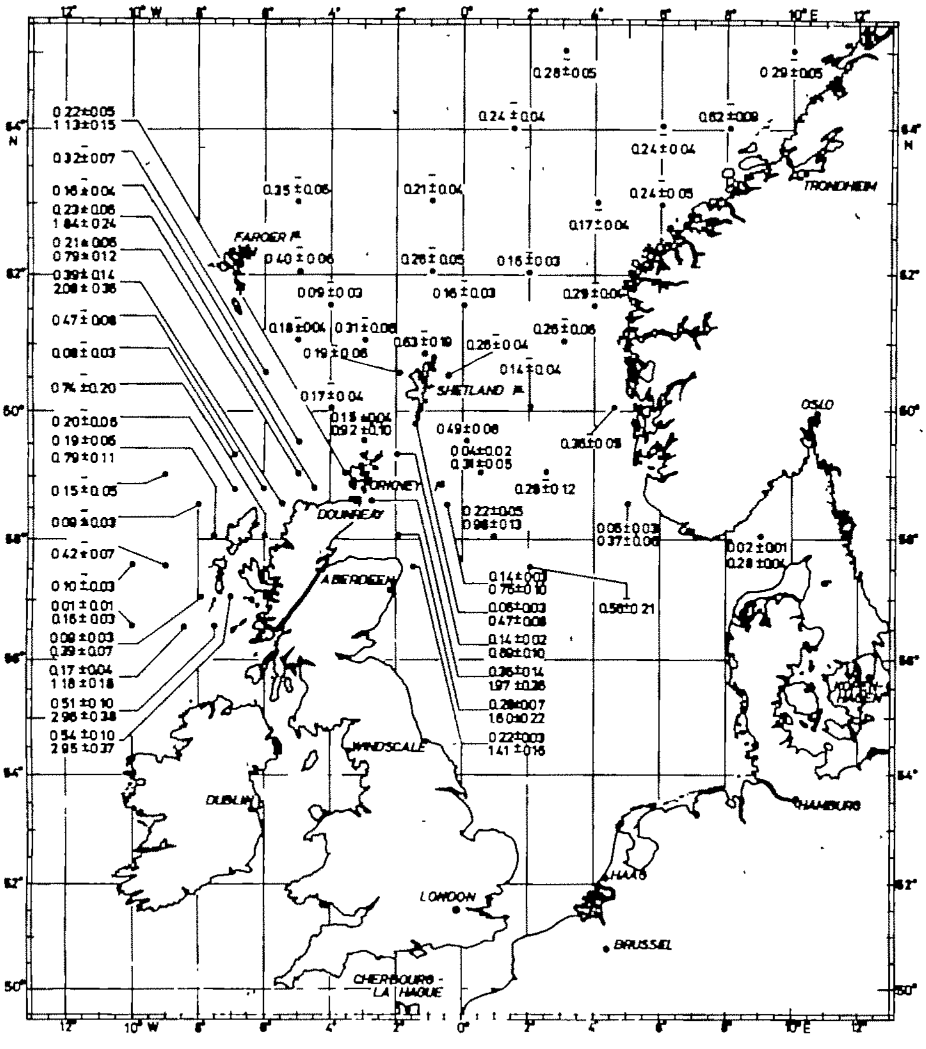


Fig. 1 ^{238}Pu, $^{239+240}$Pu: activity concentrations in Scottish coastal, North Sea and Atlantic surface waters (fCi kg^{-1}).

and 57°00′ N, 07°30′ W. One sigma (1σ) deviation from the mean values for $^{239+240}$Pu, ^{238}Pu and ^{241}Am, of three samples for plutonium and two samples for americium for each of three stations were ±14%, ±24% and ±28% at activity concentrations of the above isotopes of 2, 0.4 and 0.2 fCi kg^{-1} respectively. Results varied within the single sigma propagated error of the measurements. Reagent blanks were carried out regularly within the chemical separation scheme. All results are given with a 1σ propagated error.

Results and discussion

Figure 1 shows the surface water activity of $^{239+240}$Pu; values of ^{238}Pu are shown where they were measurable in the volumes analysed. Using the ratio $^{239+240}$Pu/^{137}Cs as a criterion for partitioning the sea area under investigation into regions of comparable ratios, three zones A, B and C were clearly distinguishable; these are shown in Fig. 2. Zone A comprises the Scottish and Norwegian coastal areas and the North Sea and has $^{239+240}$Pu/^{137}Cs ratios of $<0.5\times10^{-3}$; zone B, an intermediate area has ratios between 0.5 and 1.0×10^{-3}; and zone C, the Atlantic region, has ratios $>1.0\times10^{-3}$.

It is well documented[2,4,5] that increased ^{137}Cs activities measurable in the northern North Sea and along the east coast of the UK are due to the operation of the nuclear fuel reprocessing plants at Windscale and Dounreay.

The first increases in ^{137}Cs above fallout levels in water entering the southern North Sea through the English Channel were detected by Kautsky[2] in 1969, higher water concentrations being measured as far as the Belgian and Netherlands coastlines. The first indication in the northern North Sea was detected by Kautsky in 1971, along the northeastern coast of Scotland. Successive investigations carried out in 1971 and 1972 indicated that the caesium was being transported southwards along the east coast of England.

In water coming from the English Channel, Kautsky[2] observed that caesium was transported along the Holland and German Coasts. At this time very little northwards movement towards the southeastern coast of the UK was detected. By 1972 Kautsky[2] was able to show that some mixing of waters containing ^{137}Cs originating from UK and French fuel reprocessing plants took in the southern North Sea.

Studies by Murray and Eicke[6] in 1975 on actinide concentrations in sediment and water of the German Bight showed that they were of fallout origin, the ^{238}Pu/$^{239+240}$Pu ratio being 0.06±0.02. Data obtained from the English Channel region for 1975 Murray *et al.*[7] for ^{241}Pu showed that the apparent detectable limit of actinide intrusion into the southern North Sea, at this time originating from Cherbourg, seemed to lie around 52° N. Apparently, therefore, actinides in the northern North Sea area of the present study must be principally due to sources other than the French reprocessing plant.

Caesium concentrations in the eastern North Sea along the Danish and Norwegian coasts are probably due to mixing of waters containing ^{137}Cs originating from both UK and French reprocessing plants; however, in the central and northwestern North Sea the data of Kautsky[2,5] show that the ^{137}Cs concentrations depend most probably on the UK plants only.

Recent data by Livingston and Bowen[8] have shown that plutonium originating from Windscale is measurable in the waters and sediments of the Minch. Data have also been published by Murray and Kautsky[9] that show that increased levels of

$^{239+240}$Pu can also be measured in waters along the north-east coast of the UK and they proposed that these might also be due to the same source. In the present work, using the fact that the source of ^{137}Cs in these areas is known, statistical comparison of the plutonium and caesium data was undertaken to determine whether this source was also responsible for the actinide distribution measured in the North Sea.

Statistical considerations

The whole sea area under consideration has been divided into three major zones A,B,C shown in Fig. 2: the areas have been defined on the basis of the $^{239+240}$Pu/^{137}Cs ratio as has been previously mentioned. Because of the small number of measurements made along the Norwegian coast, the results of this region, which on the basis of its plutonium to caesium ratios belongs to zone A (Scottish coastal area and the North Sea) have not been included in the statistical analysis.

Zone A has been subdivided into two parts, west and north of the Scottish coast, and the North Sea; these are separated in Fig. 2 by a chain dotted line. For both sub-areas the relationship between $^{239+240}$Pu and ^{137}Cs is very close. As bivariate normal distributions of the data cannot be expected the rank correlation coefficient r_s (Spearman) has been taken as a measure of the closeness of the relationships. For the west and north areas of the Scottish coast, a rank correlation coefficient (r_s) of 0.95 ($\alpha < 0.001$) has been calculated; for the North Sea area the coefficient is 0.93 ($\alpha \ll 0.001$). For the intermediate area (zone B) a correlation coefficient for the relationship between $^{239+240}$Pu and ^{137}Cs of 0.78 ($\alpha < 0.005$) has been found; this is similar to that determined for stations in the Atlantic (zone C) lying south of 60°15′ N, of 0.74 ($\alpha < 0.05$). For stations in this zone lying north of this latitude a negative relationship has been estimated of −0.27 ($\alpha > 0.10$). Due to the fact that (except in the case of the North Sea area) there are tied ranks and/or samples of more than 10 pairs, the reliability of the coefficients could be only approximately determined (see ref. 10).

Judging by the nearly equal and high correlation coefficients in the two sub-divisions of zone A, it may be concluded that a major source not only of ^{137}Cs but also of $^{239+240}$Pu in these areas is the discharge of low level radioactive waste from nuclear fuel reprocessing plants in the UK; the major activity coming from the Windscale discharge (some thousands of curies) with less than 5 Ci Pu y^{-1} coming from Dounreay. The contribution of the French plant to the ^{137}Cs concentration in these areas (other than the eastern areas of the northern North Sea) is considered to be small in comparison to the UK discharges. Adjacent areas of the Atlantic also seem to be affected by the UK releases.

Further data supporting the fact that the distribution of both isotopes can be accounted for by a similar mechanism of dispersion and source, even though ^{137}Cs is in a soluble cationic form and plutonium supposedly as an insoluble poly-hydroxy-complex, were obtained using the fact that ^{238}Pu activity could also be measured in Scottish coastal and North Sea waters. Figure 3 shows the activity concentration ratios of ^{238}Pu to $^{239+240}$Pu. Visual examination of the data showed clearly that there was a strong correlation between the isotope concentrations; the rank correlation coefficient was found to be 0.96 ($\alpha < 0.001$) and thus confirmed the relationship.

To estimate the ratio ^{238}Pu/$^{239+240}$Pu a regression line of the data (Fig. 4) from the regions was calculated by the method of least squares. The resulting equation $y = 0.1756x - 0.0007$

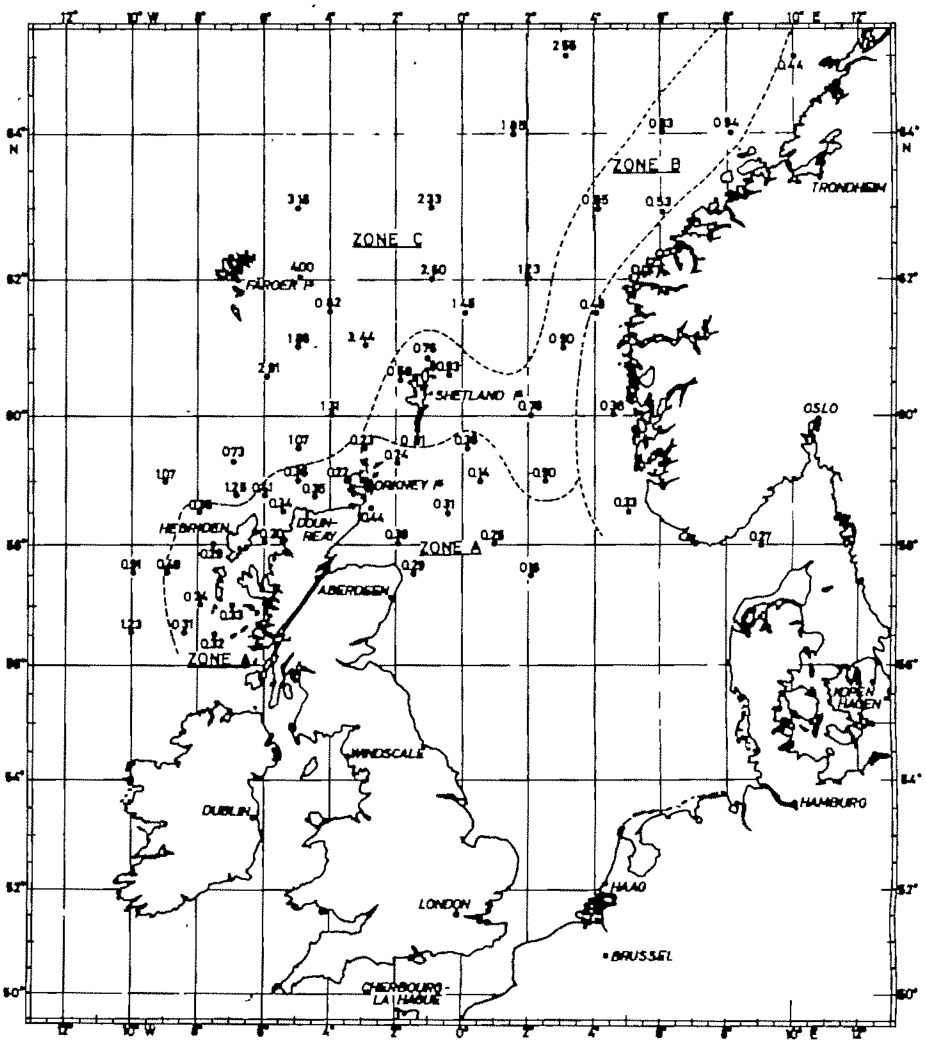


Fig. 2 Activity concentration ratios of $^{239+240}$Pu/^{137}Cs multiplied 10^3 in Scottish coastal, North Sea and Atlantic surface waters.

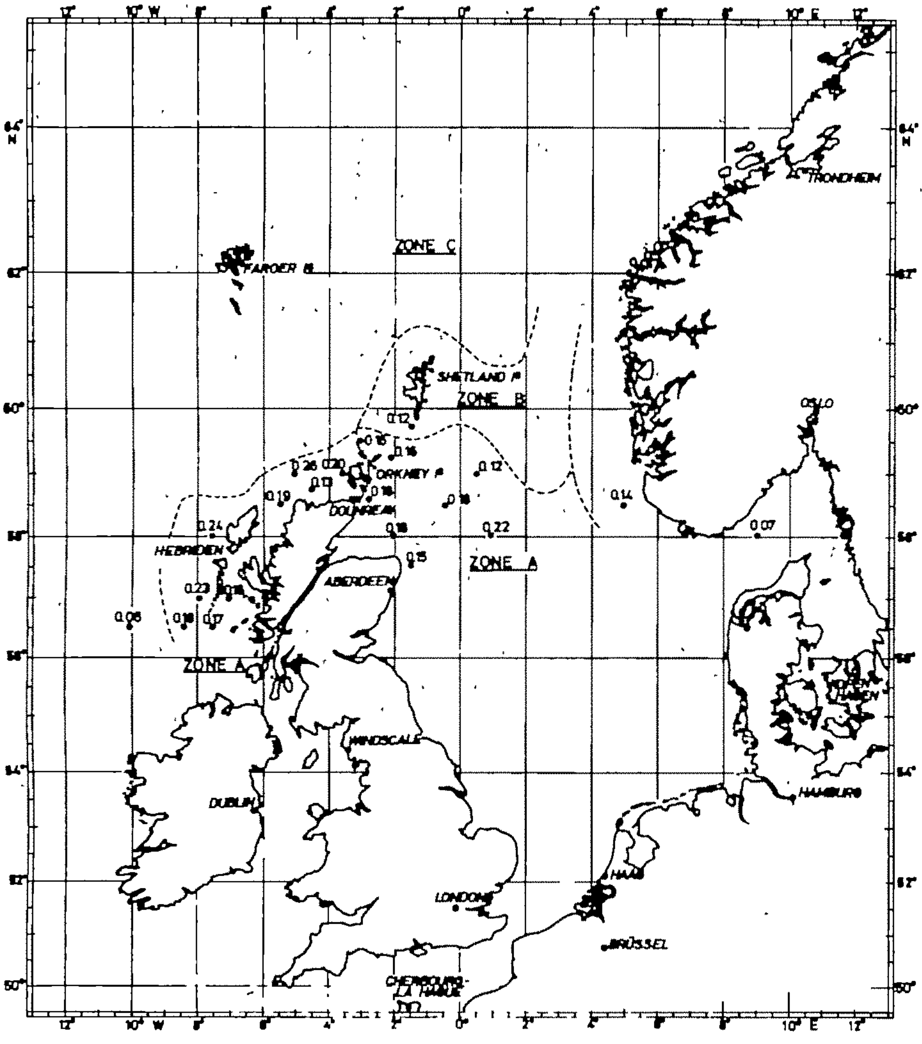
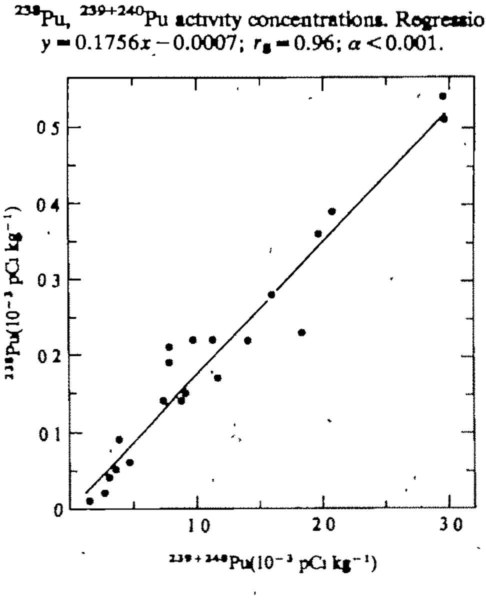


Fig. 3 Activity concentration ratios of ^{238}Pu/$^{239+240}$Pu in Scottish coastal, North Sea and Atlantic surface waters.

indicates that the regression line, as expected, goes through the origin. As both variables are subject to error the regression coefficient is distorted downwards. Estimated propagated errors have been used to calculate the correction factor for the regression coefficient according to the procedure proposed by Snedecor and Cochran[11]; the corrected coefficient was found to be 0.1797. As can be recognised, the downward bias is small. The 99% confidence limits of the regression coefficient have been estimated to be 0.1465 and 0.2129; the estimated ratio therefore of ^{238}Pu/$^{239+240}$Pu in Scottish coastal and North Sea waters is 0.18 with 99% confidence limits of 0.15 and 0.21 and is significantly different from the ratio of 0.066 ± 0.008 (1σ limits propagated error, figures calculated from data of Fukai *et al.*[12]) found for samples contaminated only from fallout plutonium in the Northern Hemisphere. Hardy *et al.*[13] report ratio results ^{238}Pu/$^{239+240}$Pu of about 0.04 for integrated samples (undisturbed soils) from the Northern Hemisphere 1970–71; the standard deviation of the average ratio being ±12%. At the 2σ level the ratios given in refs 12, 13 are indistinguishable and are significantly different from the ratios found in this present study.

Fig. 4 ^{238}Pu, $^{239+240}$Pu activity concentrations. Regression line: $y = 0.1756x - 0.0007$; $r_s = 0.96$; $\alpha < 0.001$.



It may be concluded that, as with ^{137}Cs, quantities of plutonium originating from the discharge of waste from nuclear fuel reprocessing plants into UK coastal waters are present in surface waters of the central North Sea and thus have been transported over large distances from their source of origin. An estimate of 1.1–1.8 yr for the transit time of ^{137}Cs from the vicinity of Windscale to the North Channel of the Irish Sea has been made by Jefferies *et al.*[4] for 1970–72. Two years has been estimated for the Minch by Livingston and Bowen[8]. The time required for its transport along the west and north Scottish coasts and into the North Sea is difficult to estimate, but if one takes an interval of a further 1.5–2.0 yr to pass through the Pentland Firth into the North Sea, this would mean that activities now being measured in the North Sea probably originated from discharges taking place during 1972–73 in the Irish Sea. This seems to coincide with an increased discharge by Windscale of α-containing low level waste that occurred at this time[14]. Thereafter discharges apparently decreased, according to data from Hetherington *et al.*[15,16].

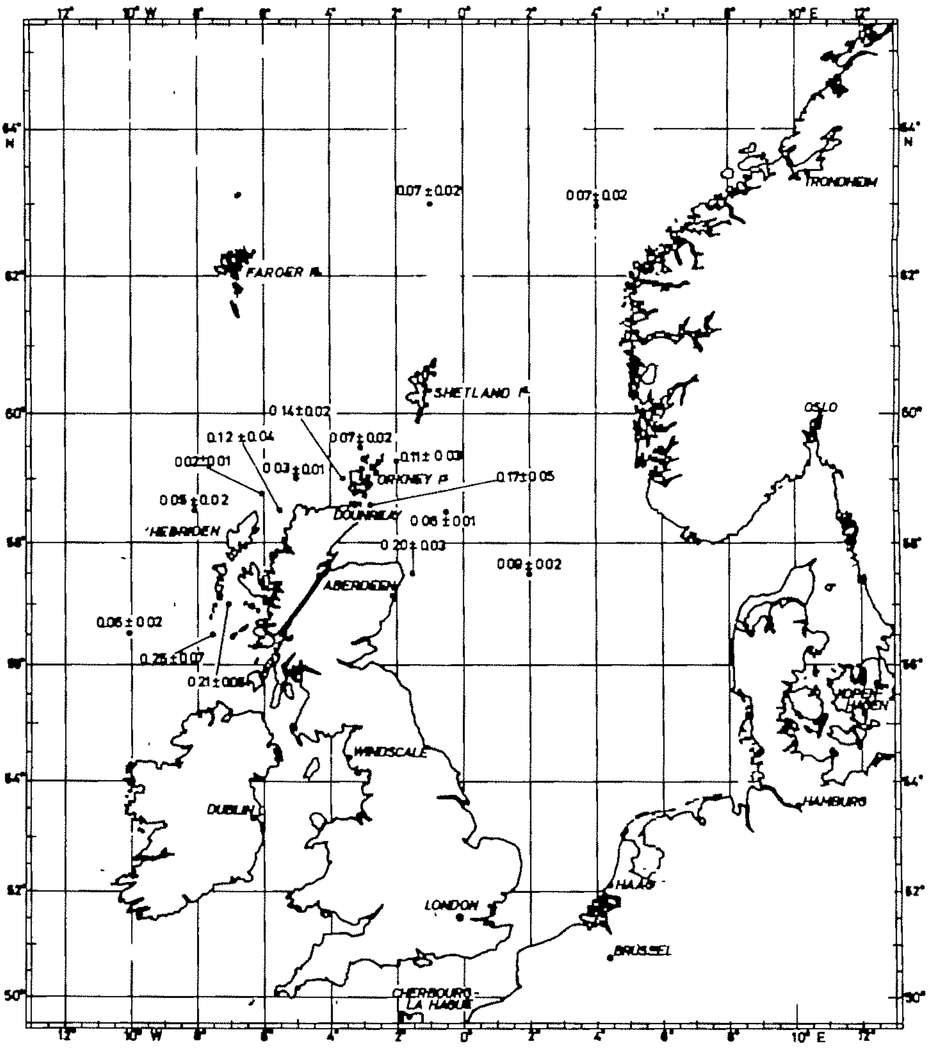


Fig. 5 ^{241}Am activity concentrations in Scottish coastal, North Sea and Atlantic surface waters (fCi kg^{-1}).

Figure 5 shows the measured activity levels of ^{241}Am in surface waters. It can immediately be seen that activities near the northwestern coast of Scotland are considerably smaller than those of plutonium; the ratio ^{241}Am/$^{238,239+240}$Pu for three stations between Barra to the Pentland Firth is only 0.06 ± 0.02. If we assume that the present results for activities in western Scottish coastal waters some 300–500 km north of Windscale are representative of discharges into the Irish Sea that occurred some 2.0–2.5 yr earlier, then this water was most probably contaminated some time in 1973–74. That these present ratios should be so small is surprising in view of the report by Hetherington *et al.*[17] who gave the Am/Pu α-radioactivity discharge from Windscale for 1971–74 as varying between 0.9 and 2.56. It seems that there has been a decrease in the ratio from about 1.7 at Windscale (average value 1971–74) to 0.06 some 300–500 km northwards along the Scottish coast. This decrease takes place in spite of the increase of ^{241}Am that will have occurred during its transport due to the decay of ^{241}Pu.

Our hypothesis that americium disappears more quickly from surface waters than plutonium is supported by data of Hetherington and Harvey[18] on the distribution of these elements between suspended material and solution in Irish Sea surface water. These workers reported that at a distance <10 km from the point of discharge, the percentage fraction of activity associated with suspended material for plutonium and americium was 36 ± 8 and 80 ± 7, respectively. The total activity in the water was found to be 2.4 ± 0.8 and 1.7 ± 0.8 pCi l^{-1} for the two isotopes. Thus at a distance of only 10 km from Windscale twice as much americium is associated with suspended material as plutonium; even at a distance of about 100 km this fraction is still more than 50%. The settling out of suspended particles from surface water thus tends to decrease the americium activity more quickly than plutonium.

In the present survey no measurable quantities of ^{242}Cm were detected in samples near the Scottish coast even though the chemical separation scheme we used is capable of detecting it at the same levels as for americium. Although the lack of ^{242}Cm activity in water could be due to the low concentrations discharged into the Irish Sea, Hetherington *et al.*[16] report it at levels similar to ^{238}Pu in the seaweed porphyra collected from the Cumberland coast. The fact that ^{242}Cm was not detected in Scottish coastal samples although apparantly present in water off the Cumbrian coast probably reflects the short half-life ($t_{1/2}$ = 163 d) of this isotope compared to the time taken for the water to travel that far north. The non-detection of curium is, however, also consistent with data obtained from the English Channel (C.N.M., unpublished data), which indicates that curium activity seems to be also lost more quickly from the water column than plutonium.

Conclusions

First, based on a statistical analysis of the distribution of ^{137}Cs and plutonium from samples obtained in July–September 1976, it has been shown that a significant fraction of surface water activity of plutonium in UK eastern coastal waters and the central North Sea originates from fuel reprocessing plants in the UK, the major contributor being Windscale.

Second, the fact that plutonium moves over such relatively great distances, although representing only a small fraction of that which is initially absorbed to bottom sediments, may have important consequences for considerations of its medium and long-term behaviour in the marine environment, especially in relation to the possible future siting of fuel reprocessing plants in North Sea coastal areas.

Finally, the apparent variations in the distribution of actinide isotopes in seawater, as shown in the present survey, underline the need for care in drawing conclusions about their environmental behaviour on the basis of a simple comparison with plutonium. The possibility of differences in behaviour for actinides coming from different sources must always be considered, when predicting their impact on the environment.

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Surface antibodies of human myelogenous leukaemia leukocytes reactive with specific type-C viral reverse transcriptases

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Purified immunoglobin G (IgG) from patients with chronic myelogenous leukaemia specifically neutralised RT from feline leukaemia virus while purified IgG from other types of leukaemias and from normal blood cells were less reactive and in some cases preferentially reacted with RT from horizontally transmitted primate type-C viruses (simian sarcoma virus–gibbon ape leukaemia virus group). This indicates the presence of a heterogeneous immune response to RT or to an RT-like molecule in humans.

SOME human leukaemic cells contain a cytoplasmic DNA polymerase similar to reverse transcriptase (RT) of RNA tumour viruses[1,2]. In some cases the enzyme has been partially purified[1,3,4]. Molecular weight, column elution, response to various template-primers and immunological tests distinguish RT from cellular DNA polymerases (α, β and γ)[1,3–8] and show its similarity to viral RT[1,3,4]. The activities of some partially purified, RT-like DNA polymerases from patients with acute myelogenous leukaemia (AML) have been specifically neutralised by IgG from hyperimmune sera prepared against RT from the simian sarcoma virus (SSV)–gibbon ape leukaemia virus (GaLV) group[3,9–12]. RT-like DNA polymerase neutralised by antisera against RT from SSV was also isolated from the spleen of a child with myelofibrosis[13] (a preleukaemic disease), who later developed AML. These results suggest that the enzymes from some AML cells and some preleukaemic cells are immunologically related to RT from these viruses Other RT-like DNA polymerases detected in human leukaemic cells were not detectably related to RT from any of the viruses studied, that is, SSV, GaLV, murine leukaemia virus (MuLV) and avian myeloblastosis virus (AMV) (refs 11, 12).

Some reports suggest expression of type-C virus-related structural proteins in human tissues (reviewed in ref. 12) and of antibodies reactive with them[14–16], but there are no reports of such interactions with purified proteins. It would be interesting to know whether antibodies to specific RTs can be identified in humans not only because of the RT-related polymerases but also because RT can be identified unambiguously by its function. We have not found anti-RT activity in human sera (unpublished results) in contrast to published results with two cases of AML[17].

We report here that the surface of some fresh human blood cells contains IgGs specifically reactive with certain viral RTs. The rationale for our experiments was (1) evidence for both the presence of a RT-like DNA polymerase in some leukaemic cells and the absence of detectable antibody to RT in human sera; (2) the precedent in solid tumours for tumour bound antibody[18] which can be eluted and isolated[19]; (3) indications of antibody on the surface of human leukaemic cells, the specific reactivity of which had not been elucidated[20,21]; and (4) indications that antibodies to RT may be present in some naturally infected cats[22], gibbons[23] and cows[24].

Detection and isolation of surface bound immunoglobulins

Leukocytes were obtained from whole blood from leukaemic or normal people by leukaphoresis or from the buffy coat of 500 ml of anticoagulated blood (acid citrate dextrose). The cells were maintained in a fresh, viable state at 4 °C during transport and washed three times at 4 °C with 10 vol. cold RPMI 1640 medium on receipt in our laboratory. They were assayed for surface Ig by measuring the binding of ^{125}I-labelled (ref. 25) F(ab')$_2$ fragments[26,27] of goat anti-human (GAH) IgG. In saturating conditions this amounted to 0.2–10 µg per 10^6 leukocytes from leukaemic donors and 0.05–0.2 µg per 10^6 leukocytes from normal donors.

The cell surface Ig could be eluted into serum-free media. The complexes formed between surface Ig and the ^{125}I-labelled GAH–IgG remained cell-bound during incubation at 4 °C overnight but were released by incubation at 37 °C in serum-free medium for 12–16 h as described by Witz[19]. The Ig detected on the surface of leukocytes was eluted from washed cells as described in the legend to Fig. 1. IgG was determined by a solid phase immunofluorescent assay (Biorad). The amounts of IgG recovered in the medium were similar to that detected on the intact cell surface by the binding assay. These findings are similar to results reported after acid treatment of leukaemic blood cells[20,21].

The Ig contained in the eluates from AML, chronic myelogenous leukaemia (CML), or normal blood leukocytes were purified as described in the legend to Fig. 1 and analysed at each step of purification by SDS polyacrylamide gel electrophoresis (PAGE). The stained gels from the crude eluates showed 5 to 10 bands, including two constituents with mobilities identical to control standard human IgG heavy and light chains. After immunoaffinity column chromatography the purified IgG on SDS PAGE revealed only two bands corresponding to heavy and light chains of IgG (not shown). The activity of the purified IgG fractions was stable at 56 °C for 1 h, non-dialysable, and not bound to DEAE-Agarose in 20 mM Na phosphate buffer, *p*H 8.

Inhibition of RT by IgG from CML leukocytes

The purified IgG was tested for inhibition of cellular DNA polymerases α, β, γ and terminal deoxyribonucleotidyl transferase (TDT), purified as before[5–8,28] from normal or leukaemic human leukocytes and for inhibition of RT purified[29,30] from various oncornaviruses. We used purified IgG because the magnitude and specificity of inhibition was greater than with the crude eluates, which contained competing proteins and protease artefacts. Cell surface IgG purified from the blood leukocytes of patients with the acute blast crisis (BC) phase of CML completely inhibited RT purified from some mammalian type-C viruses, most notably feline leukaemia virus (FeLV) (Fig. 1, Tables 1 and 2). Low concentrations of IgG were effective, for example, 50% neutralisation of FeLV RT was obtained with <0.2 μg using IgG from patient HL68 (Fig. 1*a–c*, Table 1). Table 1 shows that activity of the human cell surface anti-RT IgG compares quite favourably in specific activity with anti-RT IgG obtained from serum from animals immunised with RT or serum obtained from naturally infected animals. Furthermore, the inhibition was specific. There was no inhibition of cellular DNA polymerases α, β, γ or of TDT (Fig. 1*a*), nor of RT purified from avian type-C viruses or of RT from non-type-C retroviruses (Fig. 1*b*). Inhibition was found only with RT purified from some mammalian type-C viruses (Fig. 1*c*), especially FeLV (Fig. 1*a–c*). Blood leukocytes from eight of nine patients with CML-BC yielded antibodies with activity specifically against FeLV (Table 2). There was variation in the amount of cross-reactivity with RT from viruses related to FeLV. IgG from some patients showed remarkable type

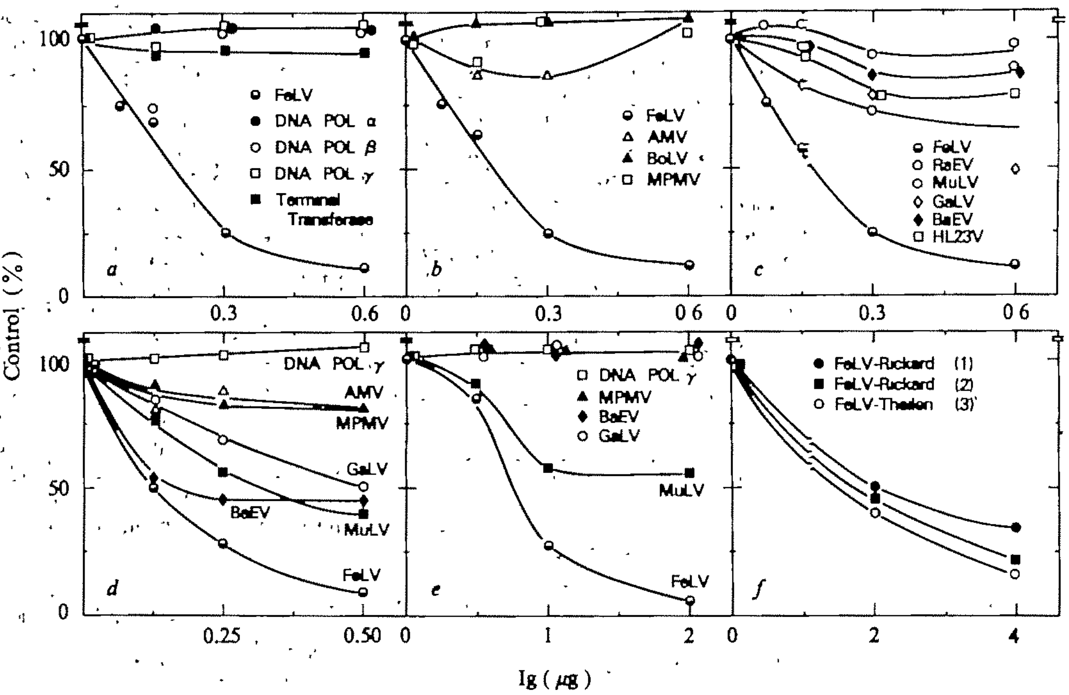

Fig. 1 Specific neutralisation of viral RT by IgG purified from blood leukocytes of patients with CML, blast crisis IgG was purified as follows. 10 g of washed blood leukocytes, obtained by leukaphoresis from patient (HL-68) with CML (BC), were incubated for 12 h at 37 °C in serum-free RPMI 1640 medium (10^7 cell ml^{-1}) containing gentamycin (50 μg ml^{-1}) and fungizone (2.5 μg ml^{-1}) to elute membrane-bound IgG. Some proteins in the eluate, including IgG, were precipitated by adding $(NH_4)_2SO_4$ to a final concentration of 50% The precipitate was dissolved in 20 mM Na phosphate, *p*H 8.0, then dialysed overnight against the same buffer An aliquot of the dialysed sample was analysed by SDS PAGE The IgG in the remaining sample, containing 4 5 mg of protein, was further purified on a DEAE Agarose column (10 mg protein per ml gel) The flow-through and 2 one-column vol washes of 20 mM Na phosphate, *p*H 8.0, were combined (total protein 1.7 mg) and further purified on an affinity column of Sepharose 4B specific for human IgG. The column was prepared as follows. Purified GAH IgG was coupled to cyanogen bromide activated Sepharose (Pharmacia in 0.1 M Na phosphate, *p*H 8.0, at a ratio of 10 mg IgG per ml 'swollen' gel The reactive groups were blocked with 0.5 M ethanolamine, *p*H 9 0, washed with 0 85% NaCl and equilibrated in solution A (0 05 M Tris–HCl, *p*H 7.4, 0.3 M NaCl containing 2% Triton X-100, 0.1% Tween 80, and BSA 10 mg ml^{-1}) One-third of the total protein of the commercial GAH IgG was specific for human IgG. Protein (1 7 mg), containing the IgG antibodies, obtained from the DEAE Agarose step was dissolved in 1 ml of solution A containing the affinity beads and incubated overnight at 0 °C. The slurry was then poured into a column, extensively washed with 0.85% NaCl until the A_{280} was < 0.01 Bound IgG was eluted with 0.2 M glycine, *p*H 2.8 and one ml fractions were collected at 4 °C. Different DNA polymerases were tested against IgG purified on DEAE Agarose and affinity chromatography. The 100% activity corresponds to activity of the different polymerases in the absence of antibody *a–c*, Purified IgG from patient HL-63, *d*, patient HL-105, *e*, *f*, patient HL-70. The IgG from patient HL-70 (*e*, *f*) were from two different preparations of purified IgG. *f* shows that RT purified from different strains of FeLV and/or by different methods are comparably neutralised by the IgG RT was purified from FeLV Rickard (1) by DEAE cellulose, phosphocellulose, poly G Agarose, and hydroxyapatite sequential column chromatography[30]. Purification of RT from FeLV Rickard (2) and for FeLV Theilen (3) was as previously described[29]. DNA polymerase (refs 5–7, respectively) and TDT[28] were purified from human leukocytes as described. FeLV (Rickard strain) was grown in feline lymphoid cells, F422; FeLV (Theilen strain) was grown in FL74 feline lymphoid cells; and various FeLV subgroups were also grown in NC37 human lymphoblasts and in canine thymus fibroblasts (FCf_2Th) AMV was obtained from chicken plasma. Other viruses are BoLV (bovine leukaemia virus) grown in sheep fibroblasts; MPMV (Mason–Pfizer monkey virus) grown in NC37 human lymphoblasts, RaEV (Rat endogenous virus) grown in Wistar rat cells; MuLV (AKR) grown in 3T3 mouse cells; GaLV (San Francisco strain), grown in NC37 cells; BaEV, grown in canine thymus cells (FCf_2Th) and in NC37 human lymphoblasts; and HL23V, a virus isolated from a woman with AML which predominantly contains a virus highly related to SSV and less of a second virus highly related to BaEV, grown in FCf_2Th cells RT were purified from different viruses after disruption in a 0 6 M KCl, 0 5% Triton X-100 by successive chromatography on DEAE cellulose, phosphocellulose, poly G Agarose and hydroxyapatite. In some cases an additional glycerol-gradient step completed purification. Details of these and alternative methods are described elsewhere[29,30]. Assays were in 0.05 M Tris–HCl buffer, *p*H 7 4 containing 140 μM ^{3}H-dTTP (3.6 Ci mmol^{-1}), 130 μM dATP, 50 μg per ml dT_{12-18} · poly A; 0.5 mM $MnCl_2$; 5.0 mM DTT; and 130 mM NaCl for all viruses with the following exceptions for AMV RT, 6 mM $MgCl_2$ was substituted for $MnCl_2$; for BoLV and MPMV RT the conditions were 50 μM ^{3}H-dTTP (10 Ci mmol^{-1}), 190 μM dATP and 40 μg per ml dT_{12-18} poly A Activities were measured at 30 °C for 30 min except for AMV, BoLV and MPMV, which were assayed at 37 °C for 30 min The enzyme and antibody were preincubated at 4 °C for 4 h in the preincubation buffer (0.05 M Tris–HCl, pH 7.4 with 130 or 190 mM NaCl, 2% Triton X-100, 10 mg per ml BSA, and 0.1% Tween 80) and then assayed as described above The 100% activity was obtained in the absence of antibody and varied between 40,000 and 70,000 c.p.m. (20–40 pmol) of ^{3}H-dTMP incorporated into acid-insoluble precipitates Comparable amounts of protein and enzyme units were used for each polymerase

Table 1 Comparison of neutralisation of cellular and viral DNA polymerases by different antibody (IgG) preparations

	Source of purified IgG with anti-RT activity†						
	Eluates from human blood leukocytes‡				Hyperimmune sera§		FeLV-infected
Source of DNA polymerase*	CML(BC) (HL-68)	CML(BC) (HL-105)	AML(HL-80)	Normal	Anti-MuLV	Anti-GaLV	cat serum‖
	(μg of IgG for 50% inhibition of polymerase)						
Human DNA pol α, β, γ and TDT	>0.6	>0.5	>10	—	—	—	—
Viral							
AMV	>0.6	>0.5	>10	—	—	—	—
MPMV	>0.6	>0.5	—	—	—	—	—
GaLV	>0.6	0.4	>10	$\boxed{0.95}$	>45	$\boxed{21}$	—
SSV	—	—	$\boxed{5}$	>5	27	42	>60
HL23V	>0.6	—	$\boxed{4}$	>5	22	>60	—
MuLV	>0 6	0 3	>10	>5	$\boxed{9}$	>60	—
BaEV	>0.6	0.25	>10	>5	—	—	>60
FeLV	$\boxed{0.15}$	$\boxed{0.125}$	>10	>5	—	—	$\boxed{15}$

* Cellular DNA polymerase (pol) γ was a 9,000-fold purified enzyme from human lymphoblasts. Similar results were obtained with DNA polymerases α, β, γ and TDT. Viral polymerases (RT) were purified as described elsewhere[29,30]. Assay conditions are described in legend to Fig. 1.

† See text and legend to Fig. 1 for IgG purification procedure.

‡ Natural antibodies eluted from human CML blast crisis, AML, and normal blood leukocytes.

§ IgG purified from hyperimmune rabbit sera obtained after inoculation of animals with purified RT from Rauscher MuLV or GaLV.

‖ IgG containing specific anti-FeLV RT activity was purified from serum derived from a cat naturally infected with FeLV

specificity for FeLV with little or no reactivity against RT from other viruses, for example, IgG from patient HL-68 (Fig. 1*c*), while IgG purified from leukocytes of other patients with CML (BC) included cross-reactivity with RT from rodent type-C viruses (patient HL-70, Fig. 1*e*) or more extensive cross-reactivity (patient HL-105, Fig. 1*d*). RT purified by different methods and from different strains (Rickard and Theilen) of FeLV were comparably neutralised by IgG from patient HL-70 (Fig. 1*f*). Therefore, the specificity in polymerase neutralisation is probably not affected by variation in specific enzyme activity among different enzyme preparations. Also, RT from FeLV grown in several different types of cells (see legend to Fig. 1) was comparably neutralised by this IgG which indicates that cellular components do not influence the specificity of the interaction.

Three separate serial blood samples were studied from patients HL-62, HL-68 and HL-101 with relatively constant clinical and haematological features. The amount of IgG required to neutralise RT and the specificity pattern for anti-RT activity were constant. Six separate IgG purifications from one blood sample (patient HL-105) and multiple purifications from others also yielded reproducible results (data not shown).

Leukocytes from CML patients in remission (two cases) were negative or contained low levels of IgG not reactive with RT. Of four patients tested with CML in the chronic phase of the disease two were negative and two contained IgG with some inhibition of RT from the GaLV-SSV group (Table 2), especially the San Francisco strain of GaLV ($GaLV_{SF}$). The IgG most reactive with FeLV RT have been predominantly from a particular type and phase of leukaemia, namely Philadelphia chromosome-positive CML-BC lacking TDT. TDT, an enzyme generally found only in certain types of lymphoblasts[31–33] was discovered in CML-BC cells[28] and subsequently found in about one-third of these patients[33], indicating that these patients may develop an acute lymphoblastic proliferation in contrast to the usual myeloblastic proliferation. The IgG with activity against FeLV RT described here appears then to be associated mainly with CML-BC with acute myeloblastic proliferation.

Table 2 Summary of human blood leukocyte surface IgG with anti-RT activity

		Anti-RT activity	
Diagnosis and disease stage	Number studied	Number positive	Specificity of inhibition†
Leukaemic samples			
CML, blast crisis*	9	8	FeLV
CML, chronic phase*	4	2	$GaLV_{SF}$
CML, remission*	2	0	—
AML, untreated	5	2	SSV (and HL23V)
AMML, untreated	4	1	SSV (and HL23V)
AUL, untreated	2	0	—
Uncertain diagnosis (AML or CML crisis)	1	1	SSV (and HL23V)
ALL	3	0	—
CLL	3	0	—
Normal samples			
PHA stimulated blood lymphocytes (pooled 12 donors)	1	0	—
Bone marrow	4	1	$GaLV_{SF}$
Buffy coat blood leukocytes	24	7	$GaLV_{SF}$

* All CML patients were Philadelphia chromosome positive. Cells from those patients in blast crisis were negative for TDT assayed as described elsewhere[28,33].

† Positive cases were those in which the RT neutralisation titration showed ≥30% inhibition and no inhibition of control enzymes (control enzymes: see Fig 1*a*, *b*)

IgG from other leukaemic and from normal leukocytes

IgG were also purified from the surface of blood leukocytes from other types of leukaemia. Antibodies reactive with RT were obtained from two of five patients with AML and one of four acute myelomonocytic leukaemia (AMML) patients (Table 2). In general, the IgGs purified from the positive AML and AMML patients mainly inhibited RT from SSV or the SSV-related virus, HL23V[34]. This was shown with intact IgG (not shown) as well as with a $F(ab')_2$ fragment derived from the purified IgG (Fig. 2*a*). The amount of IgG required to inhibit the preferred RT by 50% was much higher (5 μg) than from patients with CML-BC. There was little or no inhibition of cellular DNA polymerases or of RT from AMV, baboon endogenous virus (BaEV), GaLV, MuLV or FeLV (Fig. 2*a*, Tables 1 and 2). Similar specificity patterns were also obtained with IgG isolated from a blood sample of patient (HL-67) with an uncertain diagnosis (AML or CML-BC). The inhibition of RT by this IgG was specific for SSV and HL23V polymerases (Table 2). We also examined two cases of acute undifferentiated leukaemia (AUL), three cases of acute lymphoblastic leukaemia (ALL), and three cases of chronic lymphocytic leukaemia (CLL) for IgG with anti-RT activity—all were negative (Table 2).

There is no natural source of tissue that provides comparable control cells for human leukaemic blast cells. By using techniques described for leukaemic blood cells, we prepared eluates from various established tissue culture cell lines, for example, bat lung fibroblasts and canine thymus cells and from diverse normal human haematopoietic cells, including peripheral blood buffy coat (24 donors), bone marrow samples (4 donors) and nylon column purified PHA stimulated blood lymphocytes (pooled from 12 donors) from normal individuals. None of the tissue culture cells or the cultured human blood lymphocyte eluates contained RT inhibitory activity, but 8 of 29 fresh normal human haematopoietic cell samples exhibited significant inhibitory activity for RT from mammalian type-C viruses, particularly RT from GaLV$_{SF}$ (>40% inhibition with 5 μg of IgG). All positive results were obtained with bone marrow or with buffy coat leukocytes (Table 2). Two typical examples are shown in Fig. 2*b* and *c*. Although RT from GaLV, and SSV are known to be closely related, the IgG from normal donors and from some AML samples (Table 1) distinguished them. At antibody concentrations of 2–5 μg RT from GaLV was inhibited from 50% to 80% while RTs from SSV and HL23V were inhibited less than 20%.

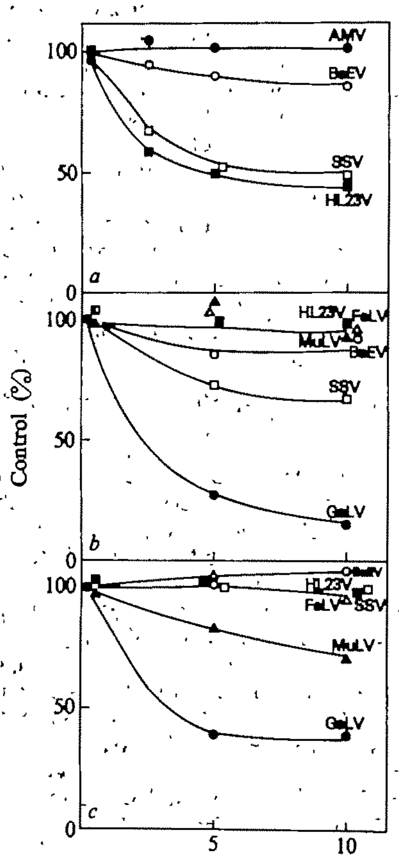

Fig. 2 Inhibition of RT purified from primate type-C viruses (SSV, GaLV, HL23V) by IgG from AML and normal leukocytes. *a*, Antibody from AML patient DEAE Agarose-purified IgG fraction from leukocyte eluates of patient HL-80 with AML was treated overnight with pepsin bound to Sepharose beads in 0.2 M NaAC buffer, *p*H 3.5. The pepsin fragments were purified on an affinity column of GAH IgG (light chain specific) The purified AML F(ab′)$_2$ fragments were then tested for inhibition of various polymerases. Cellular DNA polymerases were not inhibited (data not shown) *b*, and *c*, antibody from normal blood leukocytes DEAE Agarose-purified IgG fractions from eluates of blood leukocytes from two different normal donors were tested against different polymerases as described in text and legend to Fig. 1. Cellular DNA polymerases were not inhibited (data not shown).

Inhibition of RT is antibody mediated

Evidence indicates that the inhibitor of RT in eluates from some human leukocytes is a specific antibody and that the reaction studied is an antigen–antibody interaction. This evidence includes the biochemical and physical properties of the most active fraction, the specificity of inhibition of polymerisation reactions, the removal of known nonspecific inhibitors of these reactions and assays based on factors other than inhibition of enzyme activity. These are summarised below and in the following section.

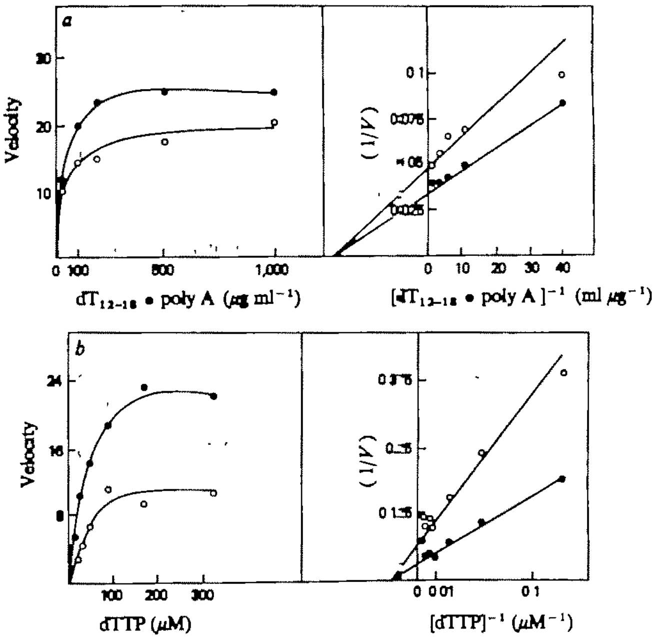

Fig. 3 Kinetics of inhibition of RT by purified IgG obtained from eluates of CML blast crisis cells (Patient HL-105) *a*, Affinity of the enzyme for the template–primer, dT$_{12-18}$ · poly A. Each 20-μl reaction mixture was preincubated for 4 h at 0 °C and contained 2 μl BaEV RT, 10 mM sodium phosphate *p*H 8, 1% Triton X-100, 0.05% Tween 80, 130 mM NaCl, 5 mg ml^{-1} BSA, with or without 10 μg of IgG (purified through the DEAE agarose step) from eluates of CML patient HL 105 The enzyme activity was then assayed at 30 °C for 20 min in a 200-μl reaction mixture containing Tris–HCl *p*H7 4, H^3-dTTP 140 μM (3 6 Ci mmol^{-1}), 130 μM cold dATP, increasing amount of dT$_{12-18}$ · poly A (25–1,000 μg ml^{-1}), 50 mM KCl, 0.5 mM MnCl$_2$. *b*, Affinity of the enzyme for substrate, dTTP. Each reaction mixture was preincubated and the enzyme assayed as in *a* except that the concentration of template–primer was constant and saturating (200 μg ml^{-1}) and the concentration of dTTP was varied [10 μM H^3-dTTP (50 Ci mmol^{-1}) and unlabelled dTTP from 0–640 μM]. Left panels show the velocity of the reaction (pmol dTMP incorporated into TCA precipitates in 30 min) as a function of the amount of template-primer (*a*) or dTTP (*b*) in the reaction with (○) and without (●) 10 μg of CML antibody. Right panels show Lineweaver–Burk representation of the same data BaEV RT was used instead of FeLV RT to avoid complete RT neutralisation. In this case the IgG cross-reacted with BaEV RT

The biochemical and physical properties of the most purified fraction indicate that the inhibitor is IgG as: the specific activity is increased by procedures known to purify IgG; in some instances after purification of the IgG fraction, F(ab′)$_2$ fractions were prepared by pepsin digestion, isolated and purified by affinity chromatography using immobilised goat IgG specific for light chains. The specificity of RT neutralisation was retained in the fraction eluting as a F(ab′)$_2$ IgG fragment (Fig. 2*a*); protein bands were observed following SDS PAGE of affinity chromatography purified IgG which coincided with IgG heavy and light chains, and the inhibitory activity was stable to heating at 56 °C for 1 h and retained after dialysis.

The inhibition of RT activity is specific. Although comparable units of polymerase activity were used, positive samples showed inhibition of only certain mammalian type-C viral polymerases without inhibition of DNA polymerases from normal cells, from avian type-C viruses, or from mammalian type-B or type-D viruses. In most cases, the inhibitory activity further discriminated between RTs from various type-C mammalian viruses. The specificity was maintained with RT purified from one type of virus grown in several types of cells, for example, IgG from CML-BC comparably neutralised RT of FeLV grown in dog, cat or human cells and without neutralising RT from other viruses grown in the same cell (see legend to Fig. 1).

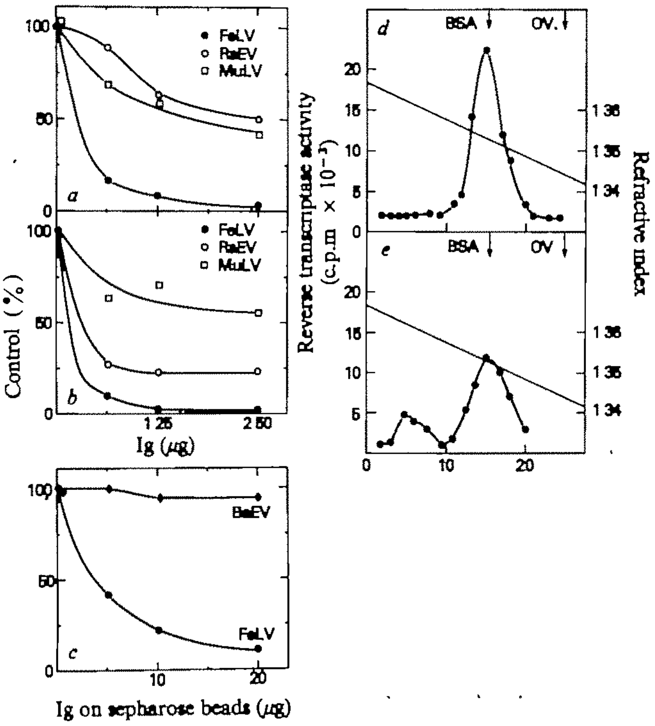

Fig. 4 Neutralisation, binding and precipitation assays for natural antibodies to mammalian type-C viral RT. Panels *a–c*, the IgG was the affinity chromatography purified IgG from blood leukocytes from CML blast crisis patient HL-70 *a*, Neutralisation of FeLV RT and cross-reaction with RT from rodent viruses Other polymerases were not inhibited (see Fig 2*e*). *b*, Indirect evidence for precipitation of RT from FeLV, murine leukaemia virus and rat type-C virus by IgG. Increasing amounts of IgG were preincubated with purified RT overnight at 0 °C in 40-μl reaction mixtures constituted as described in the legend to Fig 2. Then the samples were divided into two parts One half was assayed directly for inhibition of RT activity as shown in *a* The other half was centrifuged at 100,000 *g* for 10 min to pellet antigen–antibody complexes. The supernatant was collected and assayed for residual polymerase activity *c*, Binding of purified FeLV RT by immobilised IgG. Control normal human IgG and IgG from patient HL-70 were immobilised on cyanogen bromide activated Sepharose 4B beads. The IgG containing beads were tested for binding of purified polymerases by mixing the polymerase with the beads in a 0.05 M Tris–HCl, *p*H 7.4, containing 0.3 M NaCl, 2% Triton X-100, 10 mg ml^{-1} BSA, and 0 1% Tween 80. The mixture was incubated overnight at 0 °C, the supernatant collected after removing the beads by centrifugation, and the remaining polymerase activity in 50-μl aliquots of the supernatant was assayed as described in the legend to Fig. 1. No polymerase bound to the control normal human IgG (data not shown) which represents 100% activity. The results are % of control RT activity remaining in the supernatant *d*, Velocity sedimentation of purified SSV reverse transcriptase. Purified SSV RT was centrifuged at 40,000 r.p.m. for 16 h in a SW 50.1 rotor at 4 °C in a 5–20% linear sucrose gradient. Gradients were buffered with 25 mM Tris–HCl, *p*H 7.4, containing 10 mM sodium phosphate, 130 mM NaCl, 0 05% Tween 80 and 1% Triton X-100. Standards run simultaneously on parallel gradients were ^{125}I BSA (0 03 μg, specific activity, 75,000 c.p.m. μg^{-1}); ^{125}I-goat IgG (0.025 μg, specific activity 100,000 c.p m. μg^{-1}) *e*, Velocity sedimentation of SSV RT after incubation with natural antibody from a patient with CML-BC Method is as in *d* except that the SSV RT was incubated 4 h at 0 °C with 5 μg of purified IgG from patient HL-105 The amount of antibody used is the amount required for 70% neutralisation of SSV RT SSV RT was used instead of FeLV RT to avoid complete enzyme neutralisation

The purified IgG did not contain nonspecific inhibitors of RT. Inhibition of RT was not due to a protease which might degrade RT. The strongest evidence for this was the specificity of inhibition. In addition, the IgG fraction of the leukocyte eluates was tested for protease activity. Bovine serum albumin and purified RT from FeLV were labelled *in vitro* with ^{125}I and then incubated with either the crude leukocyte eluates, DEAE Agarose fractions or the affinity column fractions from several preparations. They were then assayed for proteases in the test IgG preparations by the release of acid soluble radioactivity or by the appearance of new radioactive peaks after velocity gradient centrifugation. As a positive control, the protein substrates were also incubated with protease K. Although the crude eluates contained protease activity, this activity was not found in the purified IgG preparations, emphasising the need to use purified IgG. Nucleases which might hydrolyse the template-primer or phosphatases which might degrade the deoxyribonucleoside triphosphate substrate were also not involved in the inhibition of RT activity. Enzyme kinetics with certain viral polymerases (known to be inhibited by a given affinity column purified leukocyte eluate) were studied with variable concentrations of template-primer at fixed and saturating concentrations of dTTP in the presence or absence of the purified IgG (Fig. 3*a*). In other experiments the concentration of dTTP was varied with a fixed and saturating concentration of primer-template with and without the IgG (Fig. 3*b*). In both experiments the V_{max} of the enzyme was substantially decreased in the presence of the inhibitor, but there was no change in K_m. This demonstrated that the inhibition was not competitive for primer-template or for substrate, indicating that inhibition was not due to contaminating nucleases or phosphatases. Furthermore, inhibition of RT by the IgG fraction was not dependent on the type of primer-template used for polymerase activity despite their variable susceptibility to nucleases. Comparable inhibitions were obtained with dT_{12-18} · poly A, poly d(A−T), dG_{12-18} · poly C and dG_{12-18} · poly C_m (data not shown). Finally, direct assays for nuclease activities were also negative. These were performed by measuring the release of radioactivity from labelled test nucleic acids in the presence of the purified IgG. Similarly, the concentrations of Mn^{2+} and Na^+ were varied to rule out the presence of a contaminating factor which might affect concentrations of cations optimal for RT activity. Again, no nonspecific effects were found. We conclude that the inhibitions directly involve RT.

Other assays for specific interaction of IgG with RT

When present in sufficient titre, specific antibodies against some viral structural proteins may directly precipitate the protein[35]. Compared with radioimmune precipitation assays, enzyme neutralisation assays combined with precipitation have the advantage of being based on the identifying characteristic of RT, that is, its enzymatic function. By incubating purified RT with the IgG overnight at 0 °C and then subjecting the sample to high speed centrifugation, RT activity present in the supernatant was quantitatively removed, suggesting that RT–IgG complexes had been precipitated (Fig. 4*b*). This precipitation was specific to only those RTs which were shown in separate experiments to be specifically inhibited by the IgG (Fig. 4*a*). Figure 4*a* and *b* also show that in addition to the complete neutralisation and precipitation of FeLV RT, RT from MuLV and rat endogenous

Table 3 Abrogation by GAH IgG of the anti-RT activity present in IgG fraction purified from peripheral blood leukocyte eluates obtained from a CML blast crisis patient

Reaction mixture*	RT activity† (pmol dTMP)	% Inhibition of RT‡
Control		
RT	7.0	0
RT+GAH IgG	6.0	0
RT+goat IgG	7.3	0
Experimental		
RT+eluate antibody (Ab)	3.1	54%
RT+(eluate Ab+GAH IgG)	5.6	16%
RT+(eluate Ab+goat IgG)	3.3	50%

* Experimental design includes assays of RT activity alone and after control preincubations with GAH IgG (50 μg) or with control goat IgG (50 μg) RT was also preincubated with 2.5 μg of IgG (here called eluate antibody) purified from eluate of blood leukocytes from patient HL-62 with CML (BC) either alone or in the presence of GAH-IgG or with control goat IgG. RT was purified from FeLV.

† RT activity expressed in pmol dTMP incorporated into TCA precipitates in 30 min (total c.p.m. = 30,000). Assay conditions are given in the legend to Fig. 1.

‡ % inhibition of RT were calculated from the mean value of controls.

type-C virus (RaEV) were partly affected by IgG from patient HL-70. Other viral and cellular polymerases were not affected (Fig. 1e). This specificity pattern is similar to the usual cross-reactivity of natural antibodies to FeLV RT present in sera from some FeLV-infected cats[22].

The natural antibody binds specifically to type-C viral RT. Purified IgG from patient HL-70, which specifically inhibited RT from FeLV and to a lesser extent RT from rodent leukaemia viruses (Figs 1e, 4a), was tested for binding to RT as follows. The IgG was immobilised on cyanogen bromide-activated Sepharose 4B beads. The beads were then incubated with various DNA polymerases, the supernatant collected after removal of the beads and the polymerase activity remaining in the supernatant determined (Fig. 4c). FeLV RT activity was specifically removed by incubation with this immobilised IgG but not with immobilised control human IgG (Fig. 4c).

We also assayed the binding of antibodies to RT by assays dependent on the physical properties of RT activity. For this purpose, the velocity sedimentation rates in sucrose density gradients were determined for RT compared to RT preincubated with purified IgG. Concentrations of antibody (patient HL-105) were used which only partially neutralised the test RT so that some enzyme activity remained to follow the position of the RT–IgG complex in the gradient. Figure 4d shows the activity of the enzyme alone and Fig. 4e the residual activity measured through the gradient in the presence of IgG. A clear shift was observed in the enzyme activity peak to a more rapidly sedimenting peak.

The RT inhibitory activity present in the IgG can be removed by GAH-IgG. If all the inhibitory activity for viral RT were due to a specific IgG antibody reaction, the inhibitory effect of the IgG would be lessened by pretreating it with an antibody to human IgG, which was the case (Table 3). Purified FeLV RT was inhibited 55% with 2.5 μg of purified antibody fraction from leukocyte eluates obtained from CML-BC patient HL-62. When the CML antibody fraction was pretreated with goat anti-human IgG, the inhibitory effect was reduced by 75%. FeLV RT was not inhibited by GAH IgG or goat IgG.

Conclusions

The results reported here show that blood and bone marrow leukocytes carry surface proteins that can be eluted into serum free medium. Among these proteins were IgG molecules reactive with specific RTs from certain mammalian type-C oncornaviruses. Among the viral RTs tested, RT from FeLV and in some instances RT from SSV, were the main DNA polymerases neutralised by IgG from blood or bone marrow cells derived from patients with myeloblastic leukaemias. The results exclude a specific interaction of the IgG with a cell protein co-purifying with viral RT as the specificity against a given RT was maintained with viruses grown in different kinds of cells from diverse species (for example, specificity of CML-BC IgG for FeLV RT with FeLV grown in either cat lymphoblasts, human lymphoblasts, or dog fibroblasts and of some AML IgG for SSV RT with SSV grown in dog thymus cells and human cells). Specificity was also maintained when different viruses were grown in the same cell (for example, a specificity of CML-BC IgG for FeLV RT when FeLV, MPMV and BaEV were grown in NC37 human lymphoblasts). Moreover, anti-RT IgG was not specifically adsorbed by uninfected cells used to grow the virus (data not shown). However, the genesis of these antibodies is unclear. It is possible that the IgG was directed against an RT which was present earlier in leukaemic and/or other cells and is now nonspecifically bound to Fc receptors on some blood cells[36] present in the leukaemic blood cell population, but this is unlikely in view of other data[20,21]. It is also possible that the IgG is directed against a cell protein, mimicking mammalian type-C viral RT. This possibility cannot be excluded until the antigen is identified and chemically characterised. However, in view of the specificities shown, we think this interpretation is unlikely and our working hypothesis is that the IgG is directed against RT present on the cell membrane either as a mature protein or in the form of a polyprotein precursor as shown with some other viral proteins[37] and recently also with RT precursors[38]. In the latter study, Witte and Baltimore reported finding RT precursors on membranes of cells infected by temperature-sensitive mutants of MuLV blocked at late stages of assembly. They did not detect enzymatically active RT except when virions were formed and released. Therefore, active RT may only be made inside released, newly made virions[38]. However, results of Wong and Kang clearly show enzymatically active viral RT in ribonucleoprotein complexes (without viral structure) present intracellularly in reticuloendotheliosis virus-transformed chicken bone marrow cells[39]. Intracisternal as well as intracytoplasmic A-particles have been repeatedly shown to contain catalytically active RT. Our experience[3,9–11] and that of others[13,40] indicates that a catalytically active RT-like DNA polymerase can sometimes be identified in some fresh human leukaemic cells and also in ribonucleoprotein complexes without demonstrable virions. In some cases of AML this polymerase immunologically cross-reacted with RT from viruses of the SSV-GaLV group[3,9–13]. These studies did not comprehensively include assays for: catalytically inactive RT, active or inactive RT in cell membranes, and FeLV or rodent virus-related RT. These studies are clearly needed in view of the recent data[38] and data reported here.

IgG may be associated with a specific type of cell, but this is not yet known because peripheral blood cells and bone marrow cells are heterogeneous even in the leukaemias. However, the absence of IgG with anti-RT activity from normal blood lymphocytes (purified by nylon column chromatography), the low or negative results with lymphoid leukaemia, combined with the positive results with some whole blood from normal donors and from patients with myeloid leukaemias, suggest that the antibodies are chiefly associated with non-lymphocytic cells.

There have been surprisingly few studies of antibodies against RT in animal models. One study in AKR mice indicates that such antibodies may be isolated from kidney eluates[41]. Serum of some animals naturally infected by retroviruses have antibodies to RT. This has recently been found in some cats infected with FeLV[22], some gibbons infected with GaLV[23] and some cows infected with bovine leukaemia virus[24] It would be of interest to determine if anti-RT IgG can be found on the surface of leukaemic blood cells of animals, particularly from non-virus producers, as a model for the results reported here.

These results provide additional evidence for the association of type-C viral information with humans, but we do not regard them as demonstrating the presence in humans of any animal virus used here These viruses were used to supply probes to detect antibodies which may have been directed against proteins derived from related information. For example, a number of other tests for FeLV components in humans carried out in our laboratory were negative, including tests for FeLV p30-related antigens in whole cell extracts of several of the samples used here and for anti-p30 activity in the leukocyte eluate IgG with anti-RT activity. Previous data obtained with probes derived from the available type-C viruses indicate that no human leukaemic blood cell sample tested contained complete provirus (of any of these viruses) in the majority of the cells[42–46] Six of the CML-BC samples shown here to contain anti-RT activity against FeLV were also tested by conventional molecular hybridisation techniques and were negative for FeLV proviral sequences, providing strong evidence against active replication of FeLV or a closely related virus in these cells. On the other hand, some non-viraemic leukaemic cats do not have detectable serum antibodies to viral proteins and proviral sequences may be difficult to demonstrate[47], despite evidence for FeLV infection as suggested by the presence of feline oncornavirus membrane antigen in the tumour cells. Also, Spiegelman reported[40] small amounts of nucleic acid sequences in all human leukaemic DNA tested which were not found in DNA from normal cells, suggesting that leukaemic cells contain acquired viral sequences and some studies indicate that the extra sequences in some cases are partly homologous to sequences of certain mammalian type-C viruses. This might be explained by the presence of incomplete provirus, by insufficient homology of the probes or by a presence of proviral sequences in a limited number of cells as described elsewhere[34]. Therefore, it seems possible that the positive findings reported here could indicate an immunological response against the test virus used. This seems especially the case with FeLV where there is both obvious contact by humans with virus-shedding cats and known ability of the virus to infect human cells[48,49]. The presence of antibody with relatively high activity and specificity to FeLV RT in CML-BC and the absence to date of IgG with similar anti-RT specificity in CML chronic phase, in CML in remission and in randomly selected normal blood samples might help in the detection of early blast transformation in this disease. Similarly, further distinction between IgG from AML and IgG from normal blood leukocytes characterised in the former case by a preferred reactivity against RT from SSV and in the latter case RT from $\mathrm{GaLV}_{\mathrm{SF}}$, may be useful in following patients in remission and in preleukaemic states.

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Nucleotide sequence of bacteriophage G4 DNA

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The 5,577 nucleotide long sequence of bacteriophage G4 DNA has been determined using the 'plus and minus' and chain termination methods of DNA sequencing. This sequence has been compared with that of the closely related bacteriophage ΦX174 (refs 1, 55). In the coding regions there is an average of 33.1% nucleotide sequence differences between the two genomes, but the distribution of these changes is not random and the sequence of some genes is more conserved than others. There is less sequence similarity between the untranslated intergenic regions of G4 and ΦX174, but despite this the sequences of the J/F, F/G and H/A untranslated spaces in both genomes have similar sized hairpin loops, which may be related to their function.

BACTERIOPHAGE G4 is a small isometric ΦX-like phage that was isolated in 1974 (ref. 2). Like ΦX174, it contains single-stranded circular DNA and the two phage genomes code for the same series of eleven proteins[2,3]. However, G4 and ΦX174 do not recombine or complement efficiently[56] and electron microscopy of ΦX174/G4 DNA heteroduplex molecules indicates that there is an overall 39% base sequence mismatch between the two DNA molecules[2]. This base sequence difference has been confirmed by the lack of similarity of the fragments obtained by restriction endonuclease digestion of ΦX174 and G4 DNA (refs 4, 5, 57). The physical maps of the two phages have, however, been aligned by detecting hybridisation between the two sets of restriction enzyme fragments[6,56].

The major physiological difference between ΦX174 and G4 is in the mechanism of DNA replication. Initiation and synthesis of the complementary DNA strand in ΦX174 requires the eight proteins dnaB, dnaC/D, dnaG, i, n, polIII holoenzyme and DNA binding protein[7,8] while in G4 it requires only three proteins, dnaG, polIII holoenzyme and DNA binding protein[9]. Also, initiation of the G4 complementary DNA strand occurs at a single unique site both *in vitro*[10] and *in vivo*[11,12], but in ΦX174 initiation of the complementary DNA strand occurs at multiple randomly located sites[13,14].

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Recently, the complete nucleotide sequence of ΦX174 has been established[1,55]. We report here the determination of the complete G4 nucleotide sequence (see Fig. 1). The G4 sequence was derived using the enzymatic priming 'plus and minus'[15] and chain termination[16] methods of DNA sequencing and extensive use was made of a computer to store and build up the G4 sequence and compare it with ΦX174. More detailed accounts of certain regions of the G4 genome and of the sequence determination are presented elsewhere[3,17,58] and a description of the computer programs used has been published[18,19].

Structure of the G4 genome

The G4 genome is 5,577 nucleotides long, 191 nucleotides longer than the ΦX174 genome[55] and, like ΦX174, it codes for 11 genes. Four of these genes specify virion structural proteins (genes *F*, *G*, *H*, and *J*), five genes specify proteins involved in viral DNA replication and phage assembly (genes *A*, *A**, *B*, *C* and *D*), one gene specifies a lysis protein (gene *E*) and the function of the new overlapping gene *K* protein[3] is not known. On SDS polyacrylamide gels (PAGE) of UV-irradiated infected cells, nine of these gene products can be identified as protein bands[2,3]. A genetic map of G4 has not been established, but a comparison of the G4 and ΦX174 nucleotide sequences indicates that the gene order in G4 is the same as in ΦX174.

Table 1 Conservation of nucleotide and amino acid sequences in the coding regions of G4 and ΦX174

Gene	Codons in G4 / Codons in ΦX174	% Amino acid changes	% Nucleotide changes	% Codon changes not changing the amino acid*	% Codons with U in the third position: G4	% Codons with U in the third position: ΦX174
A†	554/513	37.1	36.2	25.1	30.9	39.0
A*†	341/341	21.1	22.9	28.7	29.3	36.9
B	120/120	44.2	23.9	5.8	33.3	36.7
K	56/56	39.3	23.8	7.1	25.0	26.8
C†	86/84	32.6	34.5	34.9	20.2	32.6
D	152/152	17.8	25.0	39.5	41.4	42.1
E†	96/91	46.9	23.3	5.2	15.6	14.3
J†	25/38	52.6	57.0	23.7	28.0	47.4
F	427/427	34.0	33.6	31.1	42.1	52.2
G†	177/175	60.1	51.7	21.3	39.6	56.6
H†	337/328	31.6	35.7	36.8	37.1	51.8
Total	2371/2325	34.8	33.1	27.2	33.8	43.0
A(B)‡	–	27.3	23.9	25.0	14.2	15.8
A(K)‡	–	25.0	20.5	14.3	42.9	46.4
C(K)‡	–	27.6	26.7	24.1	24.1	20.7
D(E)‡	–	16.1	20.5	33.3	40.9	39.1

The changes have been calculated from the ΦX174 sequence[55] and the G4 sequence when they have been aligned for maximum homology, and take into account deletions and insertions with respect to ΦX174. In all cases the initiation codon has been included but not the termination codon. The amino acid and nucleotide mismatches are calculated by aligning the coding regions from the ATG start and comparing the sequence, counting each deleted or inserted nucleotide as a mismatch. (For the calculations that do not include the deleted or inserted nucleotides see ref 60.)

* Nucleotide changes in the third position of the codons and other conservative codon changes that do not change the amino acid. Results are expressed as: (the no. of conservative codon changes × 100)/total no. of codons.

† These genes have insertions and/or deletions with respect to ΦX174

‡ Overlapping gene regions: A(B), region of gene *A* which overlaps gene *B* (A phase); A(K), region of gene *A* which overlaps gene *K* (A phase); C(K), region of gene *C* which overlaps gene *K* (C phase); D(E), region of gene *D* which overlaps gene *E* (D phase).

In the coding regions of G4 and ΦX174 DNA, there are enough similarities in nucleotide sequence to align the two genomes and make a gene-by-gene comparison. Some of the G4 genes are slightly larger than their ΦX174 counterparts, but only in the gene *A*, *H* and *J* coding regions are there any gross changes in size (see Table 1). The larger size of the G4 genome is mainly due to an insertion of 141 nucleotides in the G4 gene *A* coding region compared with ΦX174, increased sizes of the G4 untranslated intergenic spaces (G4 has 282 or 5.0% untranslated nucleotides and ΦX174 has 217 or 4.0% untranslated nucleotides) and duplication at the 3′ end of the G4 gene *D* which introduces a 32 nucleotide long untranslated region between genes *E* and *J* that is not present in ΦX174. Also there are small insertions and deletions in G4 relative to ΦX174 elsewhere throughout the genome. A comparison of the overall structure of the G4 and ΦX174 genomes is shown in Fig. 2.

Coding regions of the G4 genome

The reading frame and starts of the G4 gene *J* and *K* coding regions[3] and gene *B*, *C*, *D*, *F*, *G* and *H* coding regions[59] were established by sequencing the first 15 to 30 N-terminal amino acids of ^{14}C-labelled G4 proteins. These proteins were isolated from SDS PAGE of UV-irradiated infected cell lysates and analysed by a radioactive microsequence method[20,21].

The phasing defined by the N-terminal amino acid sequences agrees with the phasing determined for the same genes in ΦX174 (ref. 1) and the N-terminal amino acid sequence of the G4 gene *C* protein clearly defines the start of the gene *C* coding region as overlapping with the termination codon of gene *A* (A-T-G-A) which was uncertain in ΦX174. The reading frames and initiation codons of the G4 *A* and *E* gene coding regions were established by analogy with ΦX174. The A* protein is a translational restart of the A reading frame[22] and from consideration of the size of the G4 A* protein the start of the A* coding region is most probably at nucleotide 698 (Fig. 1) which is an A-T-G codon preceded by a sequence complementary with the 3′ end of 16S rRNA (see Table 3). A correction to the ΦX174 sequence in this region[55] agrees with this prediction.

In the coding regions, the average base sequence difference between G4 and ΦX174 is 33.1%. The distribution of these changes, however, is not random and varies from gene to gene. For instance, in the non-overlapping genes 51.7% of the nucleotides in G4 gene *G* are different from their ΦX174 counterparts, while in gene *H*, 35.7% of the nucleotides are different. In the regions of the genome containing overlapping genes, the nucleotide differences are slightly lower (see Table 1).

The amino acid differences between the G4 and ΦX174 proteins again differ with the gene. Except in the case of gene *G* protein, in which 60.1% of the G4 amino acids are different from their ΦX174 equivalent, the differences in amino acids between the G4 and ΦX174 virion structural proteins (F, G, H and J) is no greater than in the non-virion structural proteins A,

Fig. 1

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A      MET
       TGCTTGACTAATACTCAATCACCACTCTAATATGCCTCCCATCAAACGGAGGCTTTTCAT
       10 20 30 40 50 60
       ↑ mRNA start? (40)

A      PHE LYS VAL HIS SER ASP TYR PHE SER LYS PRO ASN ILE ASP ALA ILE LYS PRO LEU VAL
       GTTTAAAGTACATTCTGACTACTTCAGCAAACCTAACATCGACGCAATCAAACCTCTCGT
       70 80 90 T11/16 110 120

A      GLU THR ALA GLY MET SER VAL LEU THR GLN SER PRO ASN LEU ARG ILE TRP LYS GLN CYS
       CGAAACTGCTGGTATGTCTGTTCTTACTCAATCTCCAAACCTCCGTATCTGGAAACAGTG
       T16/8 130 140 150 160 170 180

A      ASN THR ARG ILE LYS LEU LEU GLU GLU ILE LEU ALA HIS TYR THR ASN GLY ILE ARG ARG
       CAATACTCGCATCAAATTACTGGAAGAAATCCTTGCTCACTATACAAATGGTATTCGCCG
       190 200 210 220 230 240

A      ASP ASP ASN GLY ASP PHE TRP MET ASN PRO ASN SER GLN LEU ALA THR THR ILE ALA TYR
       TGACGACAATGGTGACTTCTGGATGAATCCCAACTCTCAACTCGCAACTACTATTGCCTA
       250 260 F9/5 270 280 290 300

A      ARG ALA HIS HIS LYS GLY HIS ASN PRO LYS PHE LYS GLN TYR PRO GLU THR PHE THR LEU
       TCGTGCTCATCACAAAGGCCATAACCCTAAGTTCAAACAATACCCAGAAACCTTCACTCT
       310 Z1/Za 330 340 350 360

A      ASP ASP ILE ILE THR GLY LYS PRO ILE PRO GLN THR ALA PRO ASP GLU LEU GLN LEU SER
       GGACGACATTATTACTGGTAAACCAATCCCTCAAACTGCCCCTGACGAATTACAACTCTC
       370 380 390 400 410 420

A      ASP GLU ILE VAL GLU ASP TYR ARG LEU THR VAL LEU SER ILE ILE GLU GLU LEU GLN GLU
       CGATGAAATCGTTGAAGACTATCGCCTTACAGTCCTCTCCATAATCGAAGAATTGCAAGA
       430 440 450 460 T8/10 470 480

A      CYS TYR ASP VAL LEU GLY GLN LEU ASP ILE ASN ASN THR ILE ASP HIS LYS PRO LEU GLY
       ATGCTATGACGTGCTCGGACAACTTGATATTAATAACACTATAGACCACAAACCCCTCGG
       490 500 ↑ 510 520 530 540
       Origin of viral strand replication?

A      ASN ASP HIS TRP ASN LEU LEU TYR GLU LYS PRO VAL TYR LYS HIS TRP TYR GLN LEU VAL
       TAATGACCATTGGAACCTCCTCTATGAAAAACCCGTATACAAACATTGGTATCAGCTCGT
       550 560 570 580 590 A2/4 600

A      SER ASN ARG PRO LEU LYS ASP ILE ARG ALA ASP TYR ASN TYR ALA LYS ALA LYS GLY VAL
       TTCAAATCGACCCCTTAAAGATATCCGCGCCGATTACAACTATGCAAAGGCTAAGGGTGT
       T10/12 620 H2/6 640 650 660

A,A*   LYS ASP GLU CYS SER LYS ILE LEU GLU GLU SER THR MET LYS SER ARG ARG GLY PHE THR
       TAAAGATGAGTGCTCAAAAATCTTGGAGGAGTCAACTATGAAGTCTCGACGTGGCTTTAC
       670 680 F5/11 HA/D2 ↑ 700 T12/13 710 720
       A* start

A,A*   VAL GLN ARG LEU MET ASN ALA MET ARG THR ALA HIS SER LYS GLY TRP TYR VAL VAL PHE
       CGTTCAAAGATTAATGAATGCTATGCGTACTGCCCATTCTAAGGGCTGGTATGTCGTATT
       730 740 750 760 770 780

A,A*   ASP THR LEU THR LEU ALA ASP ASP ARG LEU LYS ASP PHE TYR ASP ASN PRO ASN ALA LEU
       CGATACTCTTACTCTAGCCGATGACCGCTTAAAGGATTTCTATGATAATCCCAATGCTCT
       T13/3 790 800 810 820 830 840

A,A*   ARG ASP TYR PHE ARG ASP ILE GLY ARG MET VAL LEU THR ALA GLU GLY ARG SER VAL HIS
       TCGTGACTACTTCCGTGATATTGGTCGTATGGTGCTTACTGCCGAAGGTCGCTCGGTGCA
       850 860 870 880 890 900

A,A*   ASP SER SER SER ASP CYS TYR GLN TYR PHE CYS VAL PRO GLU TYR GLY THR GLN HIS GLY
       TGACTCATCTTCCGACTGCTATCAGTATTTTTGTGTGCCAGAGTATGGTACACAGCACGG
       F11/10 910 920 930 940 950 960

A,A*   ARG LEU HIS PHE HIS ALA VAL HIS LEU MET ARG THR LEU PRO LEU GLY SER LEU ASP PRO
       TCGTCTACATTTCCACGCAGTGCATCTTATGCGCACACTTCCTCTGGGTTCTCTGGACCC
       970 980 990 H6/13 1000 1010 1020

A,A*   ASN PHE GLY LYS LEU VAL ARG ILE ASN ARG GLN ILE ASN SER LEU GLN ASN THR TRP PRO
       TAACTTCGGTAAGCTGGTACGCATCAATCGGCAAATAAATAGCTTGCAAAACACCTGGCC
       1030 A4/12 1040 1050 1060 A12/10 1070 Z2a/9

A,A*   TYR GLY TYR SER MET PRO ILE ALA VAL ARG TYR SER GLN ASP ALA PHE SER ARG ALA GLY
       TTATGGTTACTCTATGCCCATCGCAGTCCGGTACTCCCAAGACGCATTCTCTCGCGCTGG
       1090 ↑ YA/C2 1120 1130 H13/4 1140
       mRNA start?

A,A*   TRP LEU TRP PRO VAL ASP SER LYS GLY GLU PRO LEU LYS ALA THR SER TYR MET ALA VAL
       CTGGCTCTGGCCTGTGGACTCAAAGGGTGAACCCCTTAAAGCTACCTCGTATATGGCTGT
       Z9/8a F10/7 1170 1180 A10/5 1190 1200

A,A*   GLY PHE TYR VAL ALA LYS TYR VAL ASN LYS LYS SER ASP ILE ASP MET ALA VAL LYS GLY
       AGGCTTCTACGTTGCTAAATACGTTAACAAAAAATCAGATATTGACATGGCCGTAAAAGG
       1210 1220 HD2/C 1230 1240 Z8a/10 Z10/3

B                          MET GLU GLN PHE THR GLN ASN GLN ASN GLN PRO HIS THR GLN GLU
A,A*   LEU GLY ASN LYS GLU TRP ASN ASN SER LEU LYS THR LYS ILE ASN LEU ILE PRO LYS LYS
       CCTAGGGAATAAAGAATGGAACAATTCACTCAAAACCAAAATCAACCTCATACCCAAGAA
       1270 1280 1290 1300 1310 1320
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B    SER   VAL   GLN   ASN   THR   ASN   VAL   SER   GLN   PHE   ARG   ASN   GLU   THR   VAL   ILE   ASN   GLY   SER   PRO
A,A*   VAL   PHE   ARG   ILE   ARG   MET   SER   ARG   ASN   PHE   GLY   MET   LYS   LEU   LEU   SER   MET   ALA   HIS   LEU
     A G T G T T C A G A A T A C G A A T G T C T C G C A A T T T C G G A A T G A A A C T G T T A T C A A T G G C T C A C C T
                    1330                1340                1350                1360                1370                1380

B    VAL   SER   GLY   ASN   PRO   ASP   GLY   THR   ASP   PRO   SER   GLY   LEU   ARG   ARG   ASP   PRO   VAL   GLN   GLN
A,A*   SER   ALA   GLU   THR   LEU   MET   GLU   LEU   THR   GLN   VAL   GLY   TYR   ASP   VAL   THR   PRO   PHE   ASN   ASN
     G T C A G C G G A A A C C C T G A T G G A A C T G A C C C A A G T G G G T T A C G A C G T G A C C C C G T T C A A C A A
                    1390                1400                1410                1420                1430                1440

B    HIS   LEU   GLU   ALA   GLU   ARG   GLN   GLU   ARG   ALA   GLN   ILE   GLU   ALA   GLY   LYS   GLU   ILE   CYS   ARG
A,A*   ILE   LEU   LYS   GLN   ASN   ALA   LYS   LYS   GLU   LEU   LYS   SER   ARG   LEU   ALA   LYS   LYS   SER   VAL   ALA
     C A T C T T G A A G C A G A A C G C C A A G A A A G A G C T C A A A T C G A G G C T G G C A A A G A A A T C T G T C G C
                    1450                1460         A5/1        T3/9 1480                1490                1500

B    ARG   ARG   PHE   GLY   GLY   ALA   THR   CYS   ASP   ASP   GLU   SER   ALA   LYS   ILE   HIS   ALA   GLN   PHE   ASP
A,A*   ASP   VAL   LEU   GLU   ALA   GLN   PRO   VAL   THR   THR   ASN   LEU   LEU   LYS   PHE   MET   ARG   ASN   LEU   THR
     C G A C G T T T T G G A G G C G C A A C C T G T G A C G A C G A A T C T G C T A A A A T T C A T G C G C A A T T T G A C
                    1510   H4/15        1520                1530  F7/3        1540          H15/3               1560

B    PRO   ASN   ASN   ARG   SER   VAL   GLN   PRO   THR   GLU   PHE   TYR   ARG   PHE   ASN   ASP   HIS   GLU   ILE   ASN
A,A*   ARG   THR   ILE   GLY   ALA   SER   ASN   LEU   GLN   SER   PHE   ILE   ALA   SER   MET   THR   THR   LYS   LEU   THR
     C C G A A C A A T C G G A G C G T C C A A C C T A C A G A G T T T T A T C G C T T C A A T G A C C A C G A A A T T A A C
                    1570                1580                1590                1600                1610                1620

B    LYS   TYR   GLY   TYR   PHE   ***
A,A*   SER   THR   ASP   ILE   SER   ASP   GLU   THR   LYS   ASN   TYR   VAL   ALA   SER   ALA   GLY   ILE   ALA   VAL   ALA
K                                       MET   LYS   PRO   LYS   THR   THR   LEU   LEU   LEU   GLN   GLU   LEU   LEU   LEU   LEU
     A A G T A C G G A T A T T T C T G A T G A A A C C A A A A A C T A C G T T G C T T C T G C A G G A A T T G C T G T T G C
                    1630                1640                1650                1660   P1/1      1670                1680
C                                                                                  MET   ARG   LYS   PHE   ASN   LEU   ASN
A,A*   ASN   LEU   ARG   ILE   LYS   SER   LYS   TRP   THR   ALA   GLY   GLY   LYS   ***
K        THR   TYR   GLU   LEU   ASN   ARG   SER   GLY   LEU   LEU   VAL   GLU   ASN   GLU   GLU   ILE   GLN   SER   GLN   LEU
     T A A C T T A C G A A T T A A A T C G A A G T G G A C T G C T G G T G G A A A A T G A G G A A A T T C A A T C T C A A C
                    1690      T9/14                     1710                1720                1730                1740

C    LEU   LYS   ASN   SER   ARG   SER   SER   TYR   PHE   ALA   THR   PHE   ARG   HIS   HIS   LEU   ASN   VAL   LEU   ALA
K      LYS   LYS   LEU   GLU   VAL   VAL   LEU   LEU   CYS   ASN   LEU   SER   PRO   SER   SER   GLN   ARG   ALA   GLY   LYS
     T T A A A A A A C T C G A G G T C G T C T T A C T T T G C A A C C T T T C G C C A T C A T C T C A A C G T G C T G G C A
                    1750 T14/6          1760                1770                1780                1790                1800

C    LYS   THR   ASP   ALA   LEU   ASP   GLU   GLU   LYS   TYR   LEU   ASN   MET   LEU   GLY   ALA   LEU   LEU   LYS   ASP
K          ASN   ***
     A A A A C T G A C G C C C T T G A C G A G G A G A A A T A C T T A A A T A T G T T A G G T G C T C T C C T C A A G G A C
                    1810                1820                1830                1840                1850                1860

C    TRP   PHE   ARG   TYR   GLU   GLU   HIS   PHE   VAL   HIS   GLY   LYS   GLN   SER   MET   LEU   ASP   ILE   LEU   LYS
     T G G T T C C G G T A C G A A G A A C A T T T C G T G C A T G G T A A A C A A T C A A T G C T T G A C A T A C T G A A A
                 YC2/D                  1880                1890                1900                1910                1920

C    GLU   ARG   GLY   LEU   LEU   SER   THR   SER   SER   THR   ASP   THR   ASN   HIS   LYS   GLY   ASN   ***
D                                                                                                            MET   SER
     G A A C G T G G C C T A T T A T C C A C A T C G T C A A C T G A C A C A A A C C A C A A A G G A A A C T G A A A T G T C
                 Z3/2b                  1940       RC/E 1950                1960                1970                1980
                                                 mRNA start?

D      LYS   SER   ASN   GLU   SER   ALA   VAL   ALA   PHE   GLN   THR   ALA   ILE   ALA   SER   ILE   LYS   LEU   ILE   GLN
     T A A A T C A A A C G A A T C T G C T G T A G C C T T T C A A A C T G C T A T C G C T T C T A T C A A A C T T A T T C A
                    1990   F3/1         2000                2010                2020                2030                2040

D      ALA   SER   SER   VAL   LEU   ASP   LEU   THR   GLU   ASP   ASP   PHE   ASP   PHE   LEU   THR   ARG   ASP   ARG   VAL
     A G C A T C T T C T G T T C T T G A C T T G A C A G A G G A C G A T T T T G A C T T C T T A A C T C G C G A C C G T G T
                    2050                2060                2070                2080                2090                2100

D      TRP   ILE   ALA   THR   ASP   ARG   SER   ARG   ALA   ARG   ARG   ALA   ILE   GLU   ALA   CYS   VAL   TYR   GLY   THR
E                                                                                                      MET   GLU   HIS
     C T G G A T T G C T A C C G A C C G T T C A C G C G C T C G C C G T G C T A T C G A G G C T T G C G T A T A T G G A A C
                    2110                2120  H3/8     2130        T6/4                    2150                2160

D      LEU   ASP   PHE   VAL   GLY   TYR   PRO   ARG   PHE   PRO   ALA   PRO   VAL   GLU   PHE   ILE   SER   ALA   VAL   ILE
E        TRP   THR   LEU   SER   GLY   ILE   LEU   ALA   PHE   LEU   LEU   LEU   LEU   SER   LEU   PHE   LEU   PRO   SER   LEU
     A C T G G A C T T T G T C G G G T A T C C T C G C T T T C C T G C T C C T G T T G A G T T T A T T T C T G C C G T C A T
                    2170                2180                2190                2200                2210                2220

D      ALA   TYR   TYR   VAL   HIS   PRO   VAL   ASN   ILE   GLN   THR   ALA   CYS   LEU   ILE   MET   GLU   GLY   ALA   GLU
E        LEU   ILE   THR   PHE   ILE   PRO   LEU   THR   SER   LYS   PRO   PRO   VAL   SER   SER   TRP   LYS   VAL   LEU   SER
     T G C T T A T T A C G T T C A T C C C G T T A A C A T C C A A A C C G C C T G T C T C A T C A T G G A A G G T G C T G A
                    2230                2240 RE/D1          2250                2260                2270                2280

D      PHE   THR   GLU   ASN   ILE   VAL   ASN   GLY   VAL   GLU   ARG   PRO   VAL   LYS   ALA   SER   GLU   LEU   PHE   ALA
E        LEU   PRO   LYS   THR   SER   SER   MET   VAL   LEU   ASN   ALA   PRO   LEU   LYS   PRO   LEU   ASN   CYS   SER   PRO
     G T T T A C C G A A A A C A T C G T C A A T G G T G T T G A A C G C C C C G T T A A A G C C T C T G A A C T G T T C G C
                    2290                2300                2310                2320                2330                2340

D      PHE   THR   LEU   LEU   VAL   ARG   ALA   GLY   ASN   LYS   ASP   LEU   ILE   GLY   HIS   ALA   GLU   THR   ASN   ILE
E        SER   LEU   PHE   LEU   PHE   ALA   PRO   GLU   THR   LYS   ILE   LEU   SER   VAL   THR   LEU   LYS   GLN   THR   SER
     C T T C A C T C T T C T T G T T C G C G C C G G A A A C A A A G A T C T T A T C G G T C A C G C T G A A A C A A A C A T
                    2350         H8/14  YD/C1           2370   M8/A         2380                2390                2400

D      ARG   GLU   GLN   LEU   ARG   ALA   GLN   GLY   VAL   MET   ***
E        VAL   ASN   SER   TYR   ALA   LEU   LYS   VAL   SER   CYS   LYS   ASP   LEU   ***
     C C G T G A A C A G T T A C G C G C T C A A G G T G T C A T G T A A A G A C C T T T G A T T T T A T C G T C T T C A C T
                    2410          H14/7 2420                2430                2440                2450                2460
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J                                 MET   LYS   LYS   SER   ILE   ARG   ARG   SER   GLY   GLY   LYS   SER   LYS   GLY   ALA
  T T T A A G G A G T T A T G T A A T G A A A A A A T C A A T T C G C C G C T C T G G T G G C A A A T C T A A G G G T G C
                  2470                2480                2490                2500                2510                2520

J   ARG   LEU   TRP   TYR   VAL   GLY   GLY   THR   GLN   TYR   ***
  C C G T C T C T G G T A T G T A G G C G G A A C A C A A T A C T A A T C T T T T A T G T G G G A C C G C G G T C C C A C
                  2530                2540                2550                2560                2570                2580

F                                       MET   SER   ASN   VAL   GLN   THR   SER   ALA   ASP   ARG   VAL   PRO   HIS   ASP
  T C T A T T T A A G G A T A C A A A A A T G T C T A A C G T T C A A A C A T C T G C G G A C C G T G T A C C T C A T G A
                  2590                2600                2610                2620                2630                2640

F   LEU   SER   HIS   LEU   VAL   PHE   GLU   ALA   GLY   LYS   ILE   GLY   ARG   LEU   LYS   THR   ILE   SER   TRP   THR
  C T T A T C T C A C C T T G T C T T T G A G G C T G G T A A A A T T G G C C G C C T C A A A A C T A T C T C T T G G A C
                  2650                2660                2670         Z2b/8b 2680                2690                2700

F   PRO   GLY   GLY   ALA   GLY   ASP   SER   PHE   GLU   CYS   ASP   MET   VAL   GLY   ALA   ILE   ARG   LEU   SER   PRO
  G C C T G G T G G T G C T G G T G A C T C T T T C G A G T G T G A T A T G G T T G G C G C T A T T C G T C T G T C C C C
                  2710                F1/19      T4/1 2730                2740   H7/5         2750                2760

F   LEU   ARG   ARG   GLY   LEU   ALA   VAL   ASP   SER   ARG   VAL   ASP   ILE   PHE   PRO   PHE   TYR   ILE   PRO   HIS
  T C T T C G T C G T G G C C T C G C T G T T G A C T C A C G C G T T G A T A T C T T T C C T T T C T A T A T C C C A C A
                  2770 Z8b/5b         2780 RD1/B F19/16 2790                2800                2810                2820

F   ARG   HIS   ILE   TYR   GLY   GLN   GLN   TRP   ILE   ASN   PHE   MET   LYS   ASP   GLY   VAL   ASN   ALA   SER   PRO
  C C G T C A T A T C T A C G G T C A G C A G T G G A T T A A C T T T A T G A A G G A T G G C G T T A A T G C C T C C C C
                  2830                2840                2850                2860                2870                2880

F   LEU   PRO   PRO   VAL   THR   CYS   SER   SER   GLY   TRP   ASP   SER   ALA   ALA   TYR   LEU   GLY   THR   ILE   PRO
  T C T T C C T C C T G T T A C A T G C T C C T C T G G T T G G G A C T C T G C T G C T T A T C T C G G T A C C A T T C C
                  2890                2900                2910                2920                2930                2940
                                                              F16/2                         K1/1

F   SER   SER   THR   LEU   LYS   VAL   PRO   LYS   PHE   LEU   HIS   GLN   GLY   TYR   LEU   ASN   ILE   TYR   ASN   ASN
  G T C T T C T A C C C T T A A A G T G C C T A A A T T C T T A C A T C A G G G C T A T C T G A A T A T T T A T A A T A A
                  2950                2960                2970                2980                2990                3000

F   TYR   PHE   LYS   PRO   PRO   TRP   SER   ASP   ASP   LEU   THR   TYR   ALA   ASN   PRO   SER   ASN   MET   PRO   SER
  C T A C T T C A A A C C G C C T T G G T C T G A T G A C T T A A C T T A C G C T A A C C C A T C C A A T A T G C C T T C
                  3010                3020                3030                3040                3050                3060

F   GLU   ASP   TYR   LYS   TRP   GLY   VAL   ARG   VAL   ALA   ASN   LEU   LYS   SER   ILE   TRP   THR   ALA   PRO   LEU
  T G A G G A T T A T A A A T G G G G C G T A C G T G T C G C T A A C C T T A A A T C T A T C T G G A C T G C G C C A C T
                  3070                3080                3090                3100                3110    H5/10       3120

F   PRO   PRO   ASP   THR   ARG   THR   SER   GLU   ASN   MET   THR   THR   GLY   THR   SER   THR   ILE   ASP   ILE   MET
  T C C A C C G G A T A C T C G T A C A T C T G A A A A C A T G A C T A C T G G T A C A T C C A C T A T T G A C A T T A T
          YC1/B   3130                3140                3150                3160                3170                3180

F   GLY   LEU   GLN   ALA   ALA   TYR   ALA   LYS   LEU   HIS   THR   GLU   GLN   GLU   ARG   ASP   TYR   PHE   MET   THR
  G G G C C T T C A A G C C G C A T A C G C T A A A T T A C A T A C G G A A C A G G A A C G T G A T T A C T T C A T G A C
    Z5b/4         3190                3200                3210                3220                3230                3240

F   ARG   TYR   ARG   ASP   ILE   MET   LYS   GLU   PHE   GLY   GLY   HIS   THR   SER   TYR   ASP   GLY   ASP   ASN   ARG
  C C G T T A C C G T G A C A T T A T G A A A G A G T T C G G C G G T C A T A C A T C C T A T G A T G C T G A C A A T C G
                  3250                3260                3270                3280                3290                3300

F   PRO   LEU   LEU   LEU   MET   ARG   SER   GLU   PHE   TRP   ALA   SER   GLY   TYR   ASP   VAL   ASP   GLY   THR   ASP
  T C C T C T T C T G C T C A T G C G C T C T G A A T T T T G G G C A T C T G G C T A T G A C G T A G A C G G T A C T G A
                  3310         H10/1  3320                3330                3340                3350                3360

F   GLN   SER   SER   LEU   GLY   GLN   PHE   SER   GLY   ARG   VAL   GLN   GLN   THR   PHE   ASN   HIS   LYS   VAL   PRO
  C C A A T C T T C T C T C G G T C A A T T C T C T G G T C G C G T T C A G C A G A C C T T C A A T C A C A A A G T C C C
                  3370                3380                3390                3400                3410                3420

F   ARG   PHE   TYR   VAL   PRO   GLU   HIS   ARG   VAL   ILE   MET   THR   LEU   ALA   VAL   THR   ARG   PHE   PRO   PRO
  A C G C T T C T A T G T T C C T G A G C A T C G C G T A A T T A T G A C T C T G G C G G T T A C T C G C T T T C C G C C
                  3430                3440                3450        F2/13   3460                3470                3480

F   THR   HIS   GLU   MET   GLU   MET   HIS   TYR   LEU   VAL   GLY   LYS   GLU   ASN   LEU   THR   TYR   THR   ASP   ILE
  T A C T C A T G A A A T G G A A A T G C A C T A T C T T G T A G G T A A A G A A A A C T T A A C T T A T A C C G A T A T
                  3490                3500                3510                3520                3530                3540

F   ALA   CYS   ASP   PRO   ALA   LEU   MET   ALA   ASN   LEU   PRO   PRO   ARG   GLU   VAL   SER   LEU   LYS   GLU   PHE
  T G C T T G T G A C C C T G C A C T G A T G G C T A A C C T G C C A C C T C G T G A A G T A T C C T T A A A A G A A T T
                  3550                3560                3570                3580                3590         K11/1 3600

F   PHE   HIS   SER   SER   PRO   ASP   SER   ALA   LYS   PHE   LYS   ILE   ALA   GLU   GLY   GLN   TRP   TYR   ARG   THR
  C T T C C A C T C C T C A C C C G A T T C T G C T A A A T T C A A A A T C G C T G A G G G C C A A T G G T A T C G T A C
                  3610         F13/18                     3630                3640   Z4/7b 3650                     3660

F   GLN   PRO   ASP   ARG   VAL   ALA   PHE   PRO   TYR   ASN   ALA   LEU   ASP   GLY   PHE   PRO   PHE   TYR   SER   ALA
  A C A G C C T G A C C G T G T C G C T T T C C C A T A T A A T G C T C T G G A T G G A T T C C C G T T C T A C T C T G C
                  3670                3680                3690                3700   F18/14       3710                3720

F   LEU   PRO   SER   THR   GLU   LEU   LYS   ASP   ARG   VAL   LEU   VAL   ASN   THR   ASN   ASN   TYR   ASP   GLU   ILE
  T C T C C C G T C C A C G G A A C T A A A A G A C C G T G T A C T G G T T A A C A C T A A T A A C T A C G A T G A A A T
                  3730                3740                3750         R5/A                   3770                3780
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F   PHE GLN SER MET GLN LEU ALA HIS TRP ASN MET GLN THR LYS PHE ASN ILE ASN VAL TYR
    CTTCCAGTCTATGCAGCTTGCACACTGGAATATGCAAACTAAATTTAACATTAACGTTTA
    3790  A1/7  3800  3810  3820  3830  3840

F   ARG HIS MET PRO THR THR ARG ASP SER ILE MET THR SER ***
    TCGTCACATGCCTACGACACGTGACTCAATCATGACCTCGTAACGCAACAAAGGCCGCCC
    3850  3860  F14/17  3870  3880  3890  Z7b/5a  3900

    CTCTACTGGTCAGATACCTGCCCAATGTGGGGCGGACCGTGCCTACGGAGATACTCGAGT
    3910  3920  3930  3940  3950  T1/5  F17/4

G                                                                         MET
    CTCCGATACATGGACGGCGAAAGCCGCCGTCCCTACTGCAAAGCCAAAAGGACTAACATA
    3970  3980  RNA PRIMER  3990  4000  4010  4020
    origin of complementary strand synthesis

G   PHE GLN LYS PHE ILE SER LYS HIS ASN ALA PRO ILE ASN SER THR GLN LEU ALA ALA THR
    TGTTCCAGAAATTCATTTCTAAGCACAATGCTCCAATTAACTCTACTCAGCTTGCTGCTA
    4030  4040  4050  4060  A7/13  4080

G   LYS THR PRO ALA VAL ALA ALA PRO VAL LEU SER VAL PRO ASN LEU SER ARG SER THR ILE
    CTAAAACCCCAGCTGTAGCGGCACCTGTTTTATCTGTGCCTAACTTAAGTCGCTCTACTA
    4090  A13/3  4100  4110  4120  4130  4140

G   LEU ILE ASN ALA THR THR THR ALA VAL THR THR HIS SER GLY LEU CYS HIS VAL VAL ARG
    TTCTAATCAATGCAACAACCACAGCCGTTACAACTCATTCAGGCTTGTGCCATGTTGTTC
    4150  4160  4170  4180  4190  4200

G   ILE ASP GLU THR ASN PRO THR ASN HIS HIS ALA LEU SER ILE ALA GLY SER LEU SER ASN
    GCATTGATGAAACAAACCCAACAAATCATCACGCTCTATCTATTGCAGGTTCGTTATCAA
    4210  4220  4230  4240  4250  4260

G   VAL PRO ALA ASP MET ILE ALA PHE ALA ILE ARG PHE GLU VAL ALA ASP GLY VAL VAL PRO
    ACGTCCCTGCTGATATGATTGCTTTTGCCATTCGTTTTGAGGTCGCTGATGGCGTCGTTC
    4270  4280  4290  4300  4310  4320

G   THR ALA VAL PRO ALA LEU TYR ASP VAL TYR PRO ILE GLU THR PHE ASN ASN GLY LYS ALA
    CTACTGCCGTCCCGGCCTTATACGACGTCTATCCAATCGAAACCTTCAATAACGGCAAAG
    4330  YB/E  Z5a/6  4340  4350  T5/15  4370  4380

G   ILE SER PHE LYS ASP ALA VAL THR ILE ASP SER HIS PRO ARG THR VAL GLY ASN ASP VAL
    CAATCTCATTTAAAGATGCTGTTACCATCGACTCACATCCCCGCACCGTCGGTAACGACG
    4390  4400  T15/2  F4/20  4420  4430  4440

G   TYR ALA GLY ILE MET LEU TRP SER ASN ALA TRP THR ALA SER THR ILE SER GLY VAL LEU
    TTTATCCGGGAATCATGCTCTGGTCTAACGCTTGGACCGCCTCTACTATCTCTGGCGTCC
    4450  F20/12  4460  4470  4480  4490  4500

G   SER VAL ASN GLN VAL ASN ARG GLU ALA THR VAL LEU GLN PRO LEU LYS ***
    TCTCTGTTAATCAGGTAAACCGTGAAGCAACCGTCCTTCAACCTCTGAAATAAGGATTAT
    4510  4520  4530  4540  4550  4560

H   MET PHE GLY SER ILE ALA GLY GLY ILE ALA SER ALA LEU ALA GLY GLY LEU MET GLY
    CCTATGTTTGGCTCTATCGCTGGCGGTATCGCCTCCGCACTTGCCGGAGGCCTTATGGGT
    4570  4580  4590  4600  YE/F  Z6/7a  4620

H   LYS LEU PHE GLY GLY GLY GLN SER ALA ASP SER THR GLY ILE GLN GLY ASN VAL LEU ALA
    AAATTATTTGGCGGCGGTCAGTCCGCCGACTCTACCGGAATCCAAGGCAACGTCCTTGCT
    4630  4640  F12/21  YF/A  F21/15  4670  4680

H   SER ASP ASN ASN VAL VAL GLY ALA ASN ASP ALA GLY ILE LYS SER ALA ILE GLN GLY SER
    TCCGACAACAATGTTGTAGGTGCTAATGATGCTGGTATTAAATCTGCTATTCAGGGCTCT
    4690  4700  4710  4720  4730  4740

H   THR VAL PRO ASN SER GLN GLU ALA ALA PRO SER ALA ILE SER GLY ILE LEU ALA ASP SER
    ACTGTCCCTAACTCTCAGGAAGCTGCTCCCTCTGCTATCTCTGGCATCCTTGCCGACTCT
    4750  4760  A3/8  4770  4780  4790  F15/8  4800

H   GLY LYS HIS ALA LEU SER SER LEU THR ASN ALA GLY ALA ASN LYS LEU MET GLU ALA VAL
    GGCAAGCATGCGCTCTCGTCTCTTACCAATGCTGGTGCAAATAAACTCATGGAGGCTGTC
    4810  H1/12  4820  4830  4840  4850  4860

H   GLY LEU SER LYS SER ALA SER ASP LYS GLY LYS ASP THR LYS ASP TYR LEU ALA ALA ALA
    GGCCTTTCTAAGTCTGCCTCTGACAAGGGCAAGGACACAAAAGACTATCTCGCCGCCGCA
    Z7a/1  4870  4880  4890  4900  4910  4920

H   PHE PRO GLU LEU ASN PRO TRP GLU ARG ALA GLY ALA GLY ALA SER SER PRO GLY MET GLN
    TTCCCCGAGCTCAACCCATGGGAACGTGCTGGTGCTGGCGCTTCTAGTCCAGGCATGCAA
    A8/11  4940  4950  H12/9  4970  4980

H   ASP ALA GLY PHE GLN ASN GLN LYS GLU LEU THR LYS MET GLN LEU ASP ASN GLN LYS GLU
    GACGCTGGCTTCCAAAATCAGAAGGAGCTAACCAAGATGCAGCTTGACAACCAAAAGGAA
    4990  5000  A11/14  5020  A14/6  5030  5040

H   ILE ALA LYS MET GLN ASN GLU THR GLN LYS GLU ILE ALA GLY LEU GLN SER ILE THR SER
    ATCGCTAAGATGCAAAACGAAACTCAGAAGGAAATTGCTGGGCTTCAATCTATTACATCA
    5050  5060  5070  5080  5090  5100
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H  ARG   GLU   ASN   THR   LYS   ASP   THR   VAL   TYR   ALA   GLN   ASN   GLU   MET   LEU   ALA   TYR   ASN   GLN   LYS
  C G C G A A A A T A C T A A G G A T A C T G T C T A T G C T C A A A A T G A A A T G C T C G C G T A T A A C C A A A A A
                 5110                5120                5130                5140                5150                5160

H  GLU   SER   MET   SER   ARG   VAL   GLY   ALA   ILE   LEU   GLU   ASN   THR   SER   LEU   THR   LYS   GLN   GLN   GLN
  G A G T C T A T G T C A C G C G T T G G C G C T A T T C T C G A A A A C A C A A G C C T C A C C A A A C A G C A A C A A
  ---------                             -------           -------
                 5170                5180                5190                5200                5210                5220
    F8/6                                 H9/11             T2/7

H  THR   SER   GLU   ILE   MET   ARG   GLN   MET   LEU   THR   GLN   ALA   GLN   THR   ALA   GLY   GLN   TYR   PHE   THR
  A C T T C T G A A A T T A T G C G G C A A A T G C T T A C T C A G G C T C A A A C C G C A G G T C A A T A T T T T A C A
                 5230                5240                5250                5260                5270                5280

H  ASN   ASP   GLN   ILE   LYS   GLU   LEU   THR   ARG   LYS   VAL   GLY   ALA   ASP   ILE   ASP   ALA   VAL   ARG   ALA
  A A T G A C C A A A T C A A G G A G C T T A C G A G A A A A G T T G G T G C T G A T A T T G A T G C T G T T C G C G C T
                                  -------                                                                       -------
                 5290                5300                5310                5320                5330                5340
                                   A6/9                                                                          H11/2

H  ASN   THR   ALA   ARG   THR   HIS   VAL   GLU   THR   ASP   ARG   SER   LYS   GLN   GLU   VAL   GLN   ASN   SER   ARG
  A A C A C T G C A C G C A C C C A T G T A G A A A C C G A C C G C T C A A A A C A G G A A G T A C A A A A C T C C C G T
                 5350                5360                5370                5380                5390                5400

H  TYR   ALA   SER   SER   GLN   VAL   GLY   LYS   THR   ALA   LYS   ASP   VAL   SER   ASN   ALA   ILE   THR   ASP   THR
  T A T G C C T C T T C T C A G G T A G G T A A A A C C G C T A A G G A C G T G T C C A A T G C A A T T A C T G A T A C A
                                                                                                                        -
                 5410                5420                5430                5440                5450                5460

H  ALA   GLY   SER   ILE   VAL   ASP   TYR   PHE   ARG   GLY   ALA   ASP   GLN   LYS   VAL   ALA   ASP   VAL   TYR   ASN
  G C T G G T T C T A T T G T T G A T T A T T T T C G T G G T G C T G A T C A A A A A G T G G C A G A C G T T T A C A A C
  -----                                                             -------
                 5470                5480                5490                5500                5510                5520
  A9/2                                                               MA/B

H  ASN   TYR   PHE   LYS   ASP   GLY   LYS   SER   LYS   GLY   ILE   GLU   SER   ASN   HIS   ARG   SER   LYS   ***
  A A C T A T T T C A A A G A T G G C A A A T C G A A A G G A A T A G A A T C C A A T C A C C G C T C C A A A T A A
                                            -------                 ---------
                 5530                5540                5550                5560                5570
                                             T7/11                     F6/9
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Fig. 1 Nucleotide sequence of bacteriophage G4 wild-type DNA. The sequence of the circular DNA molecule is written starting after the termination codon for the *H* gene and is numbered accordingly. The letters in the left hand margin indicate the protein whose amino acid sequence is shown in the corresponding line. Restriction enzyme recognition sites are underlined. The single letter code used for the enzymes is as follows: A, *AluI*; F, *HinfI*; H, *HhaI*; K, *KpnI*; M, *MboII*; P, *PstI*; R, *HindII*; RI, *EcoRI*; T, *TaqI*; Y, *HpaII*; Z, *HaeIII*. The G4 promoters and origins of DNA replication are marked accordingly. Nucleotides 87, 91, 1123, 2,780–2,790, 2952, and 4,096–4,098, are tentative. The amino acids are those predicted from the nucleotide sequence.

B, C, D, E and K which are presumed to have an enzymatic function in synthesis and assembly of the virus. The am acid differences between the poteins o te tw pha on in Tabl 1e *D* is the most highly conserved protein between the two phages, with only 17.8% o h msnge, uths eprtly de to the presence of the overlapping *E* gene within the gene *D* coding region (see below). The amino acid differences do not always correlate with the nucleotide differences of the coding region. This is because in some genes a high percentage of nucleotide differences are in the third degenerate position of the codon which conserves the amino acid sequence (compare gene *D* with gene *G* in Table 1).

Overlapping genes

Two overlapping gene systems have been identified in ΦX174 in which the nucleotide sequence is read in two different phases. These are gene *E* which is entirely coded within the gene *D* coding region[23] and gene *B* which is entirely coded within the gene *A* coding region[24,25]. Both of these overlapping gene systems are present in G4. The G4 gene *B* coding region has been identified by sequencing the 20 N-terminal amino acids of the G4 protein[59] and it is contained within G4 gene *A* coding region, exactly as in ΦX174. Gene *E* protein has not yet been identified on SDS PAGE either in ΦX174 or G4 and the location of the gene *E* coding region in the G4 genome has been deduced from similarities with the ΦX174 sequence (that is, the conservation of a gene *E* ribosome binding site sequence, the A-T-G initiation codon and the characteristic hydrophobic N-terminal amino acids). The G4 *E* gene coding region starts in the same position as the ΦX174 *E* gene, but it is five codons longer and, unlike ΦX174, the G4 *E* gene coding region extends beyond the end of the *D* gene (see Fig. 2). A third overlapping gene system has been identified in G4 (ref. 3). This is gene *K* which is coded as a second phase of genes *A* and *C*, crossing the junction of those two coding regions (see Fig. 2). This overlapping gene system was identified by isolating the G4 K protein and determining part of its amino acid sequence. G4 gene *K* is of particular interest because five nucleotides of its coding region are read as a third phase of pre-existing overlapping genes (see Figs 1 and 2).

The nucleotide differences between the overlapping gene regions of the G4 and ΦX174 genomes are shown in Table 1. The frequency of changed nucleotides in the G4 gene *B*, *K* and *E* coding regions compared with the same regions in ΦX174 are 23.9%, 23.8% and 23.3%, respectively. This is approximately 10–20% below the frequencies for the non-overlapping genes. However, in the three overlapping genes, fewer of the nucleotide changes are third position or conservative codon changes and this results in the gene *B*, *K* and *E* proteins having a higher than average amino acid difference between the two phages. It would appear, therefore, that there is considerable selective pressure to preserve the gene *A*, *C* and *D* amino acid sequence.

This agrees with the suggestion made previously[23] that gene *E* in ΦX174 arose at a later stage of evolution than the *D* gene and was a result of the phage utilising the high incidence of D phase codons ending in T to generate a second phase protein which was hydrophobic, as codons with T in the second position specify hydrophobic amino acids. It may be inconsistent, however, with the suggestion[24] that the present day overlapping *A* and *B* genes are a result of an evolutionary readthrough of a separate *A* gene into a pre-existing *B* gene coding region.

The most noticeable feature of the gene *E* and *K* proteins is their strongly hydrophobic N terminus. Both G4 gene *E* and *K* proteins have 10 leucine residues in the first 25 N-terminal amino acids and these are distributed in two blocks in a manner similar to certain secretory precursor proteins[3,26]. This suggests that the most important feature to preserve in these proteins is their hydrophobic N terminus and the amino acid sequence of the rest of the molecule is more free to drift in evolution.

Thus in the overlapping coding regions of G4 and ΦX174, the frequency of nucleotide change is only slightly lower than in the non-overlapping coding regions and the increased number of amino acid changes in the overlapping gene proteins appears to be accommodated by the lack of stringency in the amino acid sequence. Use of the DNA sequence in two concurrent reading frames need not, therefore, be a significant barrier to evolutionary change

Codon use

G4 DNA does not have the same high T content as ΦX174 DNA and its base composition as obtained from the nucleotide sequence is A, 27.17%, C, 25.93%, G, 19.80%, and T, 27.11%. This compares with the ΦX174 base composition of A, 23.95%, C, 21.48%, G, 23.30% and T, 31.27% (ref. 55). The high frequency of codons with T in the third position that was noted in ΦX174 (ref. 27) is not so marked in G4. Overall, 33 8% of the G4 codons have T in the third position compared with 43.0% in ΦX174. A gene-by-gene analysis of the number of codons with third position T is shown in Table 1. It is noticeable that in the overlapping gene regions of the genome, the percentage of codons with T in the third position is high and almost the same in both G4 and ΦX174, but in the non-overlapping gene regions which code for the structural virion proteins, although the incidence of third position T is still high in ΦX174, it is much lower in G4.

The spectrum of codon use in G4 is similar to that in ΦX174. This is shown in Table 2 and is compared with the codon use in MS2, which is the only prokaryotic virus for which extensive information is available[28]. Some codons are rarely used in G4. For example, out of a total of 123 Arg codons, only 4 and 6 are A-G-G and A-G-A respectively, and out of 139 Gly codons, only five are G-G-G. The figures are similar in ΦX174. In general, codons ending in G-G and G-A are rare in both G4 and ΦX174. These rare codons may have a modulating role in translation if their corresponding tRNA is also rare, as suggested by Fiers *et al*[28] The use of codons by these three prokaryotic viruses is considerably different from the use of codons by the eukaryotic virus SV40 (refs 29, 30).

Most of the G4 and ΦX174 genes have the same termination codons, except for genes *F* and *G*, which use T-A-A in G4 and T-G-A in ΦX174. The A-T-G-A overlapping termination codon of gene *A* and initiation codon of gene *C* is present in both genomes, but the two other overlapping ΦX174 termination and initiation codons (genes *C*/*D* and *D*/*J*) are not present in G4.

Untranslated regions

There are untranslated sequences between most of the G4 coding regions and two of these (between G4 genes *E* and *J* and genes *C* and *D*) are not present in ΦX174. This is shown in Fig. 2. One of the new G4 intergenic spaces is a result of a duplication of the 3′ end of the overlapping G4 gene *D* and *E* coding regions which introduces a 32–nucleotide untranslated sequence between the *E* and *J* genes[55]. The other new intergenic space has been introduced by changes in the end of the G4 gene *C* coding region relative to ΦX174, which has resulted in a loss of two gene *C* codons and an insertion of one untranslated A nucleotide between the G4 *C* and *D* genes. In G4 the sequence T-G-A-A-A-T-G has replaced the overlapping gene *C* and *D* termination and initiation codons (T-G-A-T-G) present in ΦX174 (Fig. 1).

The sequences of the H/A and J/F intergenic regions are about 50% conserved between the G4 and ΦX174 genomes when they are aligned for maximum homology. The large F/G and the small G/H intergenic regions, however, show little sequence homology between the two bacteriophages. Despite the sequence differences, the three large intergenic spaces in both G4 and ΦX174 (J/F, F/G and A/H) can form hairpin loops which are in the same relative position in the sequence and have similar size and geometry. This is illustrated in Fig. 4 which shows the untranslated sequences between G4 and ΦX174 genes *H* and *A*. The similarity of the 3′ end of the sequence is a special case because of the presence of a ribosome binding site, a promoter and an mRNA termination site. The conservation of the hairpin loops in the J/F and H/A untranslated regions is believed to be related to the transcriptional termination sites observed in these regions in ΦX174 (see below).

The G4 untranslated region between genes *G* and *F* contain three hairpin loops[17] compared with two in ΦX174 (ref. 31) and one of these in G4 is associated with the origin of G4 complementary DNA strand synthesis. The function of the other hairpin loops in this region in G4 and ΦX174 is not known.

G4 promoters and mRNA termination sites

There is little information on transcription and translation in G4. Three G4 RNA polymerase binding sites have been identified by filter binding experiments[32] and these appear to correspond with the three RNA polymerase binding sites observed in analogous experiments in ΦX174 (ref. 33). Two of the ΦX174 RNA polymerase binding sites have been shown by mRNA studies[34–37] to be the gene *A* and gene *D* promoters, but

Fig. 2 Aligned physical genetic maps of bacteriophage G4 and ΦX174. The G4 and ΦX174 genetic maps are drawn to scale from the G4 (Fig. 1) and ΦX174 (refs 1, 55) nucleotide sequences The lengths of the untranslated regions are shown above the intergenic spaces The locations of the three promoters (P_A, P_B and P_D) and the origins of viral DNA strand synthesis (O_V) and complementary DNA strand synthesis (O_C) are described in the text. The position of the nucleotides inserted and deleted in the G4 coding regions relative to ΦX174 have been deduced from an alignment of the complete G4 and ΦX174 nucleotide sequences.

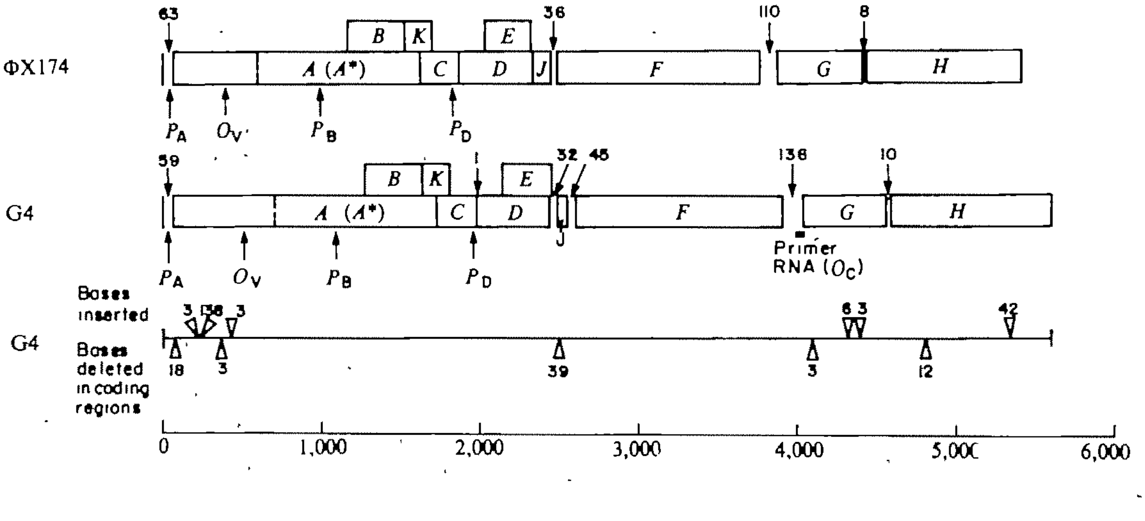

Table 2 Use of codons in G4, ΦX174 and MS2

		G4	ΦX174	MS2			G4	ΦX174	MS2			G4	ΦX174	MS2			G4	ΦX174	MS2
Phe	TTT	33	71	19	Ser	TCT	86	65	15	Tyr	TAT	49	60	9	Cys	TGT	9	15	6
	TTC	61	37	29		TCC	27	19	20		TAC	36	21	32		TGC	9	15	6
Leu	TTA	37	32	17		TCA	40	23	16	Ter	TAA	5	3	1	Ter	TGA	6	8	0
	TTG	20	50	11		TCG	21	25	22		TAG	0	0	2	Trp	TGG	33	31	23
Leu	CTT	53	67	15	Pro	CCT	48	46	17	His	CAT	34	30	6	Arg	CGT	46	56	21
	CTC	44	22	26		CCC	19	11	10		CAC	23	11	9		CGC	47	45	20
	CTA	16	11	15		CCA	21	9	9	Gln	CAA	64	48	17		CGA	12	12	10
	CTG	43	35	9		CCG	16	25	12		CAG	35	62	22		CGG	8	10	11
Ile	ATT	51	75	12	Thr	ACT	59	66	19	Asn	AAT	63	73	17	Ser	AGT	12	16	8
	ATC	57	22	25		ACC	46	27	21		AAC	80	43	28		AGC	6	10	16
	ATA	10	7	19		ACA	38	21	13	Lys	AAA	110	91	19	Arg	AGA	6	16	7
Met	ATG	72	76	20		ACG	19	35	14		AAG	44	56	26		AGG	4	3	6
Val	GTT	51	83	21	Ala	GCT	97	123	26	Asp	GAT	48	79	28	Gly	GGT	63	75	37
	GTC	36	17	21		GCC	39	32	21		GAC	81	62	22		GGC	48	45	16
	GTA	26	15	16		GCA	34	20	21	Glu	GAA	74	36	16		GGA	23	23	12
	GTG	30	20	21		GCG	16	26	23		GAG	31	65	28		GGG	5	5	16

the third binding site is in gene *G* where no mRNA is known to start.

The third ΦX174 promoter—that preceding the *B* gene—has been identified in the ΦX174 nucleotide sequence (ref. 1 and J. Sims and D. Dressler, personal communication). The similarity of the G4 sequence and ΦX174 sequence in the regions of the ΦX174 promoters suggests that the three G4 promoters are in the same location as in ΦX174. The sequences of the ΦX174 gene *A*, *B* and *D* promoters with the analogous G4 sequences are shown in Fig. 3. Note that all six sequences preserve the common feature of having a sequence similar to T-A-T-Pu-A-T-Pu centred about eight nucleotides from the mRNA initiation point[38,39] and a sequence similar to T-G-T-T-G-A-C-A-A-T-T-T centred about 35 nucleotides from the mRNA initiation point (refs 40, 41 and J. Majors, unpublished). The 5′ end of the major G4 mRNA species can therefore be predicted as A-U-C-A-A (gene *A*), G-U-A-A-C (gene *D*) and A-U-C-G-C-A (gene *B*).

Table 3 Proposed ribosome binding sites in G4

Gene	Nucleotide sequence
A	A-T-C-A-A-A-C-$\boxed{\text{G-G-A-G-G}}$-C-T-T-T-T-C-$\boxed{\text{A-T-G}}$
A*	A-T-C-T-T-$\boxed{\text{G-G-A-G-G}}$-A-G-T-C-A-A-C-T-$\boxed{\text{A-T-G}}$
B	A-G-G-C-T-$\boxed{\text{A-G-G}}$-G-A-A-T-A-A-A-G-A-A-$\boxed{\text{A-T-G}}$
K	C-A-A-G-T-A-C-$\boxed{\text{G-G-A}}$-T-A-T-T-T-C-T-G-$\boxed{\text{A-T-G}}$
C	G-T-G-G-A-C-T-G-C-T-G-G-T-$\boxed{\text{G-G-A}}$-A-A-$\boxed{\text{A-T-G}}$
D	A-C-C-A-C-A-$\boxed{\text{A-A-G-G-A}}$-A-A-C-T-G-A-A-$\boxed{\text{A-T-G}}$
E	C-T-A-T-C-$\boxed{\text{G-A-G-G}}$-C-T-T-G-C-G-T-A-T-$\boxed{\text{A-T-G}}$
J	C-T-T-T-$\boxed{\text{T-A-A-G-G-A-G}}$-T-T-A-T-G-T-A-$\boxed{\text{A-T-G}}$
F	C-T-A-T-T-$\boxed{\text{T-A-A-G-G-A}}$-T-A-C-A-A-A-A-$\boxed{\text{A-T-G}}$
G	A-G-C-C-A-A-$\boxed{\text{A-A-G-G-A}}$-C-T-A-A-C-A-T-$\boxed{\text{A-T-G}}$
H	T-G-A-A-A-$\boxed{\text{T-A-A-G-G-A}}$-T-T-A-T-C-C-T-$\boxed{\text{A-T-G}}$
16S rRNA 3′ end	$_{\text{HO}}$A-U-U-C-C-U-C-C-A-C-U-A-G

The main mRNA termination site in ΦX174 has been identified at the 3′ end of a T-T-T-T-T-T-A sequence located at the base of the hairpin loop in the untranslated region between the *H* and *A* genes (J. Drouin, unpublished) (Fig. 4). Similar T_6A sequences have been found at the 3′ end of RNA transcripts in phage λ (refs 42, 43) and in bacteriophage fd (ref. 44), and in both these cases it has been suggested that the hairpin loop preceding the T_6A sequence is an important signal for transcriptional termination. In G4 there is a T-T-T-T-C sequence instead of a T-T-T-T-T-T-A at the base of the hairpin loop in the H/A untranslated region (Fig. 4) and from analogy this is most probably the major G4 mRNA termination site.

A minor ΦX174 mRNA termination site has been identified to the 3′ side of the hairpin loop in the J/F untranslated region (J Drouin, unpublished). This hairpin loop is also conserved in G4 but with no sequence homology, and it is not known whether there is a transcriptional termination site in this position in G4

G4 Ribosome binding sites and possible new overlapping proteins

Table 3 shows the sequences preceding the A-T-G initiation codons of known G4 coding regions. The nucleotides complementary to the 3′ end of 16S RNA which are characteristic of ribosome binding sites[45] are indicated. The five G4 ribosome binding sites that occur in the untranslated intergenic spaces (those for genes *A*, *J*, *F*, *G* and *H*) have different sequences from the same sites in ΦX174 whereas the G4 ribosome binding sites that occur in coding regions of the preceding gene (those for genes *A**, *B*, *K*, *C*, *D* and *E*) are almost identical to their ΦX174 counterpart.

In G4 there appear to be other sites which could code for a protein. There are 13 sites in the genome which have complementarity with the 3′ end of 16S ribosomal RNA and are located within 14 nucleotides of an A-T-G initiation codon and followed by an open phase of at least 20 codons (Table 4). Only two of the G4 potential initiation sites are present in ΦX174 (which has others that are not represented in G4 (ref. 46)) and would specify the same length protein. These are the G4 A2a (and A2b in the same phase) and F2 potential proteins (Table 4). It is not known whether any of these sites are used to synthesise a so far undetected protein *in vivo*, but one new G4 overlapping gene, ***K***, was predicted in this manner from the nucleotide sequence and the protein was subsequently found[3].

Origin of G4 viral DNA strand synthesis

G4 has a unique origin of synthesis of its viral and complementary DNA strands which are separated and on almost opposite sides of the genome (Fig. 2). The *in vivo* origin of the G4 viral DNA strand has been located by pulse label experiments[47,11] and by electron microscope mapping of the 5′ end of the tail of late rolling circle replicative intermediates[48]. It is in the *Haemophilus aegyptius* restriction endonuclease fragment *Hae*III2b near the junction of the *H. influenzae* restriction endonuclease fragment *Hin*dIIA and D. When the G4 and ΦX174 sequences are compared across this region[17], there are 30 nucleotides (500–529 in Fig. 1) which are identical in both genomes and within this sequence the ΦX174 cistron A protein is known to cleave ΦX174 double-stranded DNA and initiate viral DNA strand synthesis[49]. This cleavage is between the G and A nucleotides which correspond to G4 nucleotides 506 and 507 in Fig. 1. On the 5′ and 3′ sides of the conserved sequence there are considerable differences between the G4 and ΦX174 sequences but in both cases the cistron A cleavage site is in an A-T-rich sequence (501–523 in G4) flanked by G-C-rich sequences (490–500 and 524–541 in G4). The conservation in G4 of this feature and the sequences around the ΦX174 cistron A protein cleavage site strongly suggest that the G4 origin of viral DNA strand synthesis is in the same place as in ΦX174, between G4 nucleotides 506 and 507.

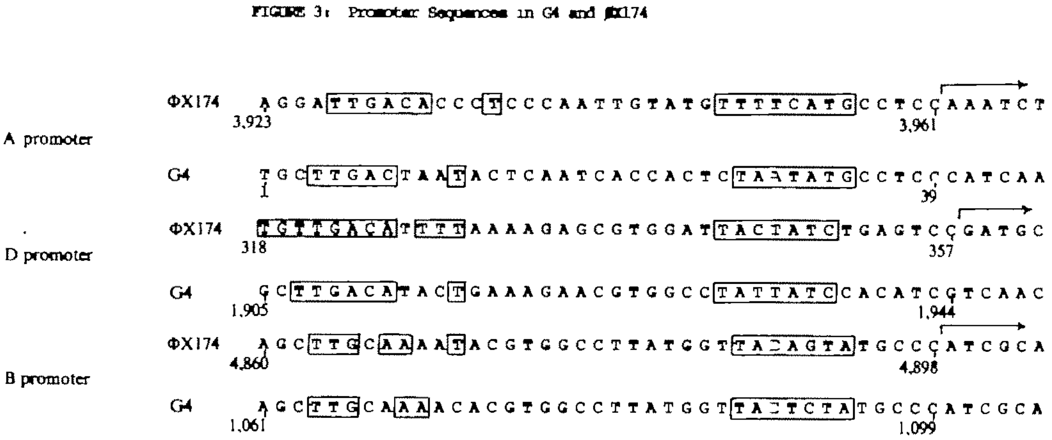
FIGURE 3: Promoter Sequences in G4 and ØX174
A promoter
ΦX174 A G G A T T G A C A C C C T C C C A A T T G T A T G T T T T C A T G C C T C C A A A T C T
3,923
3,961
G4 T G C T T G A C T A A T A C T C A A T C A C C A C T C T A A T A T G C C T C C C A T C A A
1
39
D promoter
ΦX174 T G T T G A C A T T T T A A A A G A G C G T G G A T T A C T A T C T G A G T C C G A T G C
318
357
G4 G C T T G A C A T A C T G A A A G A A C G T G G C C T A T T A T C C A C A T C G T C A A C
1,905
1,944
B promoter
ΦX174 A G C T T G C A A A A T A C G T G G C C T T A T G G T T A C A G T A T G C C C A T C G C A
4,860
4,898
G4 A G C T T G C A A A A C A C G T G G C C T T A T G G T T A C T C T A T G C C C A T C G C A
1,061
1,099

Fig. 3 Promoter sequences in G4 and ΦX174 The ΦX174 promoter sequences are taken from Sanger *et al.*[1] and the ΦX174 nucleotide numbering is taken from the corrected ΦX174 DNA sequence[55]. The boxed regions indicate homology with T-A-T-Pu-A-T-Pu[38,39] and T-G-T-T-G-A-C-A-A-T-T-T (refs 40, 41 and J Majors, unpublished) The locations of the ΦX174 mRNA starts are shown with arrows.

Origin of G4 complementary DNA strand synthesis

G4 has single unique origin of synthesis of its complementary DNA strand, both *in vitro*[9] and *in vivo* (refs 11, 12). Initiation *in vitro* involves synthesis of an RNA primer molecule[10] the sequence of which has now been determined[50]. This 28-nucleotide RNA sequence corresponds to a G4 DNA sequence in the untranslated region between genes *F* and *G* (ref. 17 and J. Sims and D. Dressler, personal communication) and the 5′ end of the primer RNA is at nucleotide 3,997 in Fig. 1. The DNA corresponding to the RNA primer sequence can be drawn in the form of a stable hairpin loop (positions 3,972–3,992 in Fig. 1). Two other hairpin loops are formed in the G4 region between genes *F* and *G* and these appear to be analogous to the loops observed in this region in ΦX174 (ref. 31). ΦX174 does not have a loop corresponding to the G4 RNA primer and there is no evidence for a preferred initiation site for complementary DNA strand synthesis in ΦX174.

It is interesting to note that the sequence of 42 nucleotides on the 3′ side of the primer RNA loop in G4 (before the 5′ start of

Fig. 4 Untranslated region between genes *H* and *A* The ΦX174 nucleotide sequence is from ref. 1 and the identification of the *A* promoter sequence, the ribosome binding site and mRNA termination sites are discussed in that paper. The G4 promoter ribosome binding sites are deduced from analogy with the ΦX174 nucleotide sequences (see text)

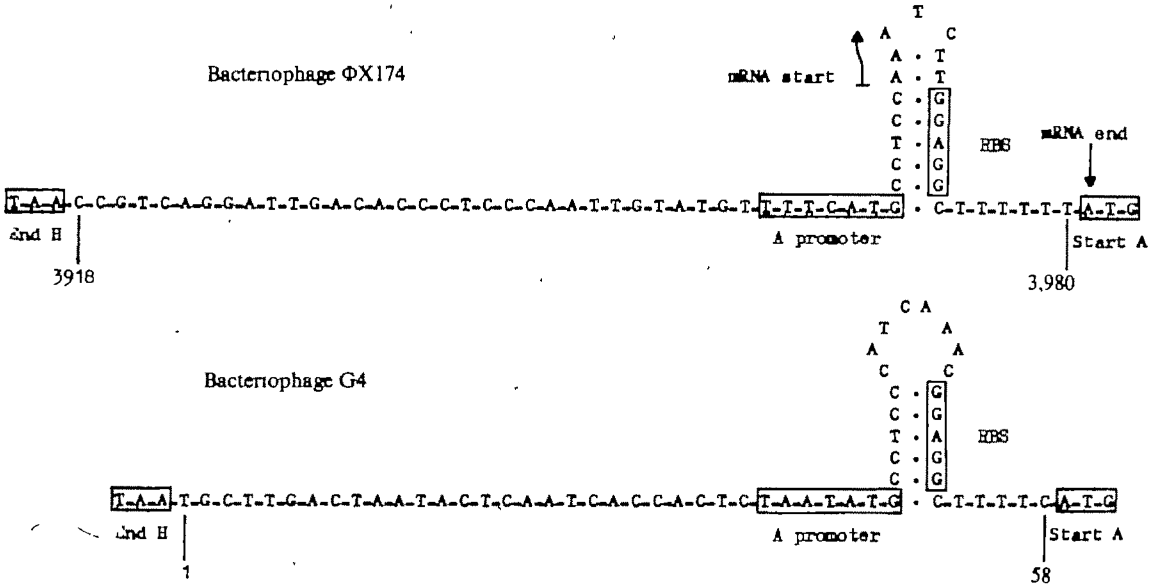
Bacteriophage ΦX174
mRNA start
mRNA end
RBS
T-A-A-C-C-G-T-C-A-G-G-A-T-T-G-A-C-A-C-C-C-T-C-C-C-A-A-T-T-G-T-A-T-G-T-T-T-T-C-A-T-G · C-T-T-T-T-T-T-A-T-G
End H
3918
A promoter
Start A
3,980
Bacteriophage G4
RBS
T-A-A-T-G-C-T-T-G-A-C-T-A-A-T-A-C-T-C-A-A-T-C-A-C-C-A-C-T-C-T-A-A-T-A-T-G · C-T-T-T-T-C-A-T-G
End H
1
A promoter
Start A
58

Table 4 Possible extra protein initiation sites in G4

Name*	Position†	Sequence‡	Length of protein§
A1	564	C-A-T-T-$\boxed{\text{G-G-A}}$-A-C-C-T-C-C-T-C-T-$\boxed{\text{A-T-G}}$	45
A2a	822	C-G-C-T-T-A-$\boxed{\text{A-A-G-G-A}}$-T-T-T-C-T-$\boxed{\text{A-T-G}}$	78
A2b	900	G-A-$\boxed{\text{A-G-G-T}}$-C-G-C-T-C-G-G-T-G-C-$\boxed{\text{A-T-G}}$	52
F1	2637	G-C-$\boxed{\text{G-G-A}}$-C-C-G-T-G-T-A-C-C-T-C-$\boxed{\text{A-T-G}}$	74
F2	2871	A-T-G-$\boxed{\text{A-A-G-G-A}}$-T-G-G-C-G-T-T-A-$\boxed{\text{A-T-G}}$	38
F3	3285	G-G-C-$\boxed{\text{G-G-T}}$-C-A-T-A-C-A-T-C-C-T-$\boxed{\text{A-T-G}}$	21
G1	3970	C-T-C-$\boxed{\text{G-A-G}}$-T-C-T-C-C-G-A-T-A-C-$\boxed{\text{A-T-G}}$	42
G2	4192	C-A-T-T-C-$\boxed{\text{A-G-G}}$-C-T-T-G-T-G-C-C-$\boxed{\text{A-T-G}}$	28
G3a	4309	C-G-T-T-T-T-$\boxed{\text{G-A-G-G-T}}$-C-G-C-T-G-$\boxed{\text{A-T-G}}$	68
G3b	4444	G-T-C-$\boxed{\text{G-G-T}}$-A-A-T-C-G-C-G-T-T-T-$\boxed{\text{A-T-G}}$	24
H1	4706	A-A-T-G-T-T-G-T-$\boxed{\text{A-G-G-T-G}}$-C-T-A-$\boxed{\text{A-T-G}}$	101
H2	5126	A-C-T-$\boxed{\text{A-A-G-G-A}}$-T-A-C-T-G-T-C-T-$\boxed{\text{A-T-G}}$	78
H3	5444	G-C-T-$\boxed{\text{A-A-G-G-A}}$-C-G-T-G-T-C-C-A-$\boxed{\text{A-T-G}}$	36
	16S rRNA	$3'_{\text{HO}}$A-U-U-C-C-U-C-C-A-C-U-A-G 5′	

* Sites are named according to which gene they occur in and the order in which they occur in that gene. Sites labelled a, b, etc. are in the same reading frame.

† Numbers refer to the A of the A-T-G initiation codon.

‡ Only those sequences that have at least three consecutive nucleotides complementary to the boxed sequence at the 3′ end of the 16S rRNA are included. Other restrictions are that these complementary sequences must not be closer than two nucleotides and not further than 14 nucleotides from the A-T-G, and must have at least two potential G/C base pairs with the 16S sequence shown boxed. Possible extra initiation sites in the same reading frame as known genes are not included nor are sites containing G-T-G initiation codons.

§ Length of the polypeptide that could be produced starting at the A-T-G initiation codon. Only those sites that could specify a polypeptide of greater than 20 amino acids have been considered.

the primer RNA) has 26 nucleotides in common with phage λ origin of DNA synthesis[51]. This common sequence, together with the hairpin loops that are present in both organisms, may represent the dnaG protein (primase) binding site. The equivalent sequence in ΦX174 has only 11 nucleotides in common with G4.

Location of the *Eco*RI and *Eco*B restriction sites in G4

G4 grows only on *Escherichia coli* C strains and is restricted by K12 and B strains and by strains carrying the plasmid RY (ref. 52). The single *Eco*RI restriction enzyme cleavage site (G-A-A-T-T-C) is at position 3,596 (Fig. 1) and the single *Eco*B restriction site (T-G-A-8N-T-G-C-T)[53,54] is located between positions 4,523 and 4,537 on the complementary strand. Neither of these sites is present in ΦX174.

Evolution of G4 and ΦX174

The comparative G4 and ΦX174 nucleotide sequence presents an opportunity to analyse the mechanism of evolution simultaneously on both the nucleotide and amino acid levels. It is clear that G4 and ΦX174 are closely related and have the same overall genome structure and organisation of gene function. The main nucleotide differences between the two genomes are most probably explained by single step mutational nucleotide changes and the increased size of the G4 genome (+3.5%) could probably have taken place by duplications of the sort that are still evident at the end of the G4 gene *D* (nucleotides 2,420–2,434 containing the gene *J* ribosome binding site and initiation codon and the gene *D* termination codon are repeated at positions 2,463–2,477). However, any evolutionary description will have to take into account the following observations: (1) the intergenic untranslated regions of G4 and ΦX174 are different in size and sometimes quite different in nucleotide sequence; (2) both insertions and deletions in one genome relative to the other occur in single gene coding regions (genes *A*, *G* and *H*) and occur in units of triplets so that the amino acid phasing is not changed; (3) alternative serine codons (A-G-C to T-C-T) are used in one phage relative to the other in regions of highly conserved amino acids (for example, at positions 1,186 and 1,304 in gene *A*). An A-G-C to T-C-T change cannot take place in single steps without changing the amino acid. These changes may have taken place by the known recombinational mechanisms acting on the pool of replicating viral DNA or even within a single genome (that is, intramolecular recombination) rather than deletions and insertions on codon unit lengths of DNA.

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letters to nature

Positions and identifications for galactic X-ray sources 2A1822−371 and 2S1254−690

PRECISE positions and optical identifications for the galactic X-ray sources 2A1822−371 (4U1822−37) and 2S1254−690 (4U1254−69), are presented here using the scanning modulation collimator (MC) on HEAO 1 (ref. 1). Briefly, the HEAO 1 MC instrument contains two independent four-grid collimator systems, one having planes of maximum transmission with 30 arc s. FWHM, and the other having 120 arc s FWHM, the overall field of view of each being limited to 4°×4° FWHM. The planes of transmission of the modulation collimators are inclined at ±10° to the scan direction, so that the lines of position from each have an included angle of 20°. Data are folded and binned around the catalogued positions[2–4] of X-ray sources in the manner described by Gursky *et al.*[1].

2A1822−371($l^{\mathrm{II}}=356°$, $b^{\mathrm{II}}=-11°$): data were accumulated in scans across 2A1822−371 for a total of 59 orbits, from 25 to 29 September 1977 UT, when the source was within ±2° of the HEAO 1 scan circle. The source was detected at 14.7σ above background in the 30 arc s collimator (MC1) and at 13.5σ in the 2 arc min collimator system (MC2). The strength of the source was measured as 6 UFU (1 Uhuru flux unit $=1.7\times10^{-11}$ erg cm^{-2} s^{-1}, 2–6 keV). This is to be compared with maximum catalogued intensities of about 25 UFU[2,3,6] and a minimum of 6 UFU[3]. The present observations were thus taken when the source was weak. Of the multiple possible positions obtained by the instrument, only one falls within either the 2A or 4U error boxes for the X-ray source, this one MC position (of area 0.6 arc min^2) being entirely within the 2A box (50 arc min^2) and on the edge of the 83 arc min^2 4U box. The HEAO MC position for 2A1822−371 is summarised in Table 1.

The first step in identifying the optical counterpart of this source was to carry out multicolour photographic photometry of the above HEAO MC location. The Cerro Tololo Inter-American Observatory 1 m telescope was used on 28–30 April 1978 with the Carnegie Image tube camera. B and R filters were used which combine with the S20 photocathode to give a spectral response closely approaching the Johnson standard colours (the U filter was not used in this case because of scattered moonlight). Enlargements of the B and R plates are shown in Fig. 1, with the HEAO MC X-ray source location overlaid. The star marked S is clearly extremely blue when compared with all neighbouring stars. Subsequent spectrophotometry of star S was carried out 3–4 May UT, using the CTIO 4 m telescope and silicon intensified target (SIT) vidicon, providing digital readout. The very blue nature of the spectrum was readily confirmed, but accurate photometric magnitudes were difficult to ascertain because of poor seeing conditions. However, we estimate magnitudes of $V\sim16.29$ and $B\sim16.24$, with errors of 0.25, and a colour index $(B-V)=-0.05\pm0.1$. Intense emission lines of the λλ4640–4650 CIII/NIII blend and λ4686 He II were easily visible in ~10-min observations, with measured equivalent widths of 1.9 Å for CIII/NIII and 2.8 Å for He II. A

Table 1 HEAO 1 MC positions (1950.0) for 2A1822−371 and 4U1254−69

	2A1822−371		4U1254−69	
	RA	dec	RA	dec
Centre	18h22min22.92s	−37°08′09″	12h54min15.84s	−69°01′20″
90% confidence corners	−2″.3	−69″	+50″	+49″
	+12″.6	+1″	+8″	−9″
	2″.6	+70″	−50″	−49″
	−12″.1	−1″	−9″	+9″
Optical counterpart (positional uncertainty 4″)	18h22min22.7s	−37°08′3″	12h54min20.9s	−69°01′07″
	star 'S'		star '30'	

systematic uncertainty ±10% in these equivalent widths arises from the estimation of the continuum level. Given the location of this star within the HEAO MC error box, the very blue nature of the spectrum and the presence of emission lines characteristic of X-ray binary systems (see ref. 5), we consider the identification of this X-ray source to be secure.

As the X-ray spectrum of this source has been shown to be relatively hard (energy spectral index $\alpha \sim 0.7$, with hydrogen column density $N_H \sim 8.10^{22}$ atoms cm^{-2}) (ref. 6), a search for X-ray and/or optical pulsations may be worthwhile.

4U1254−69[3]($l^{II} = 303°$, $b^{II} = -6°$): data from 4U1254−69 (2S1254−690) were accumulated early in the HEAO mission, from 24 orbits between 17 and 19 August 1977 UT. The source was detected at 12σ above background in MC1 and 14σ in MC2, with a measured flux of ~6 UFU (2–6 keV), to be compared with previously catalogued values of 23.2 UFU (2–6 keV) (ref. 3) and ~25 UFU (2–11 keV) (ref. 4). The source was thus considerably weaker than in previous observations; variability is clearly indicated by a factor of 4, as reported from OSO 7 observations[6]. This galactic source has been accurately positioned by SAS 3 and Fig. 2 shows the overlay of our MC error box (diamond) and the 25 arc s radius SAS 3 circle[4] on our CTIO 1 m B plate. The coordinates for the MC error box for 4U1254−60 are shown in Table 1.

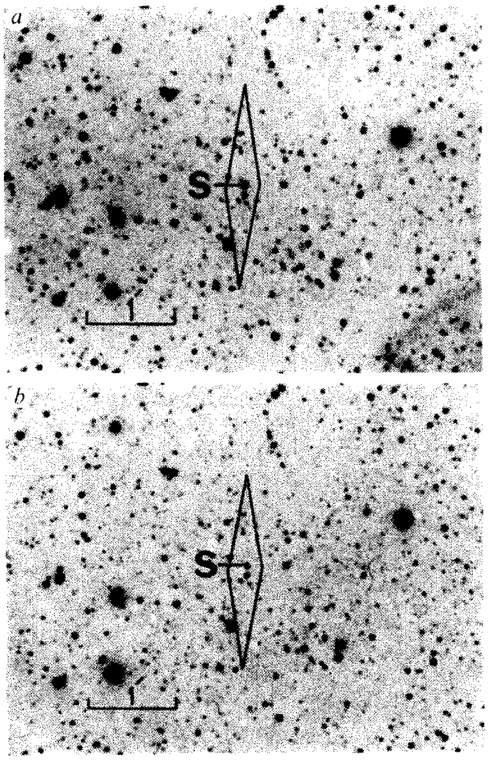

Fig. 1 Finding chart for optical counterpart to 2A1822−371. HEAO MC position (diamond), shown superimposed on our CTIO 1 m B (*a*) and R (*b*) plates. Star S is the optical candidate, discussed in the text. North is up and east to the left is both Figs 1 and 2.

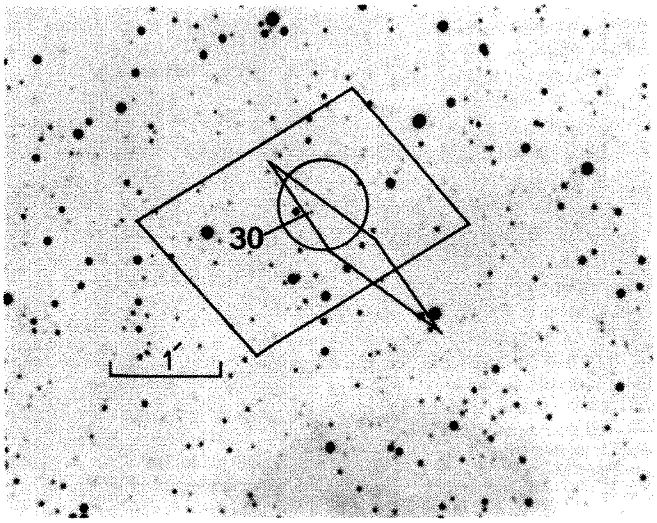

Fig. 2 Finding chart for the optical candidate to 4U1254−69, taken from our CTIO 1 m B plate. The HEAO MC position is the diamond, the circle is the SAS 3 position[4], and the rectangle is the 4U position[3]. The optical candidate is the star labelled '30'.

Using the same equipment described above, U, B and V plates of the 2S1254−690 field showed star 30 of Dower *et al.*[4] to be relatively blue compared to other stars. Estimated magnitudes from our Vidicon observations are $V = 19.09 \pm 0.1$ and $B = 19.24 \pm 0.1$. Spectra of star 30 were taken on 2 and 3 May 1978 UT, which were summed together (~2.7 h integration time) to reveal the λλ4640–4650 CIII/NIII blend with an equivalent width of 5.4 ± 1 Å. As star 30 is within both the SAS 3 and HEAO MC error boxes, we again consider this star as a probable optical identification.

Because of the strong UV excesses observed in these two objects, and their relatively high galactic latitude, neither is likely to be substantially reddened. Thus, it is extremely unlikely that they are early type systems (for example, OB supergiants such as Cyg X-1) as this would imply a distance of ~100 kpc and corresponding X-ray luminosities $\sim 10^{39}$ erg s^{-1}. Given the low reddening, we may compare their X-ray-to-optical luminosity ratios, L_X/L_{opt} with those of other known X-ray counterparts. Taking the bolometric corrections to be zero for simplicity and consistency, and converting to energy units[7], we find L_X/L_{opt} to be ~600 for 4U1254−69 and ~25 for 2A1822−371. Using the same procedure, Sco X-1 has an L_X/L_{opt} of ~900. (The observed colour of 4U1254−69 is very similar to that of Sco X-1; indeed, it may be even more blue.) Thus, 4U1254−69 is remarkably similar to Sco X-1 in this respect, whereas 2A1822−371 is different by more than an order of magnitude. Bolometric corrections are unlikely to account for this effect as the colours of the candidate objects are broadly similar. We suggest that much of the optical luminosity of 2A1822−371 originates in the normal star. The smaller equivalent width of the CIII/NIII blend in 2A1822−371 supports this interpretation.

Assuming the same luminosity for 4U1254−69 as for Sco X-1 ($\sim 4 \times 10^{36}$ erg s^{-1}, 2–10 keV) and a distance to Sco X-1 of 500 pc, we may estimate the distance of 4U1254−69 to be ~10 kpc. As far as we can tell from present observations this system has an optical spectrum similar to that of the steady component of the optical–X-ray burster MXB 1735−44 (refs 8, 9), and colours similar to those of the 7.7 s optical–X-ray pulsating source 4U1626−67 (refs 10, 11).

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Interpretation of tails of X-ray bursts

ALCOCK AND HATCHETT[1] recently proposed that the tails of X-ray bursts[2,3] observed during burst decay (time scales typically tens of seconds) are due to the small-angle scattering of the original pulse of X rays on interstellar dust grains. To produce the desired tails, and obey constraints set by optical interstellar reddening data, their model uses grains with a typical size of ~3 μm. Here we give four independent reasons which rule out their model (see also ref. 3).

(1) Bursts from one source in general are similar to one another[2,4]. However, a burst is sometimes detected with a tail much longer (already pointed out by Alcock and Hatchett) or shorter than is typical for that source. This has been observed for example in bursts from MXB1837+05 (SAS-3, unpublished data) and MXB1728−34 (ref. 5) where the minimum duration of the tails was a few seconds, the maximum ~100 s.

Because of the low interstellar reddening of the globular cluster NGC6624, Alcock and Hatchett conclude that the tails of the bursts from MXB1820−30 in this cluster cannot be explained by their model but are intrinsic to the source. Additional evidence for this conclusion is contained in a series of bursts from MXB1820−30, reported by Clark *et al.*[6]. The tails in the bursts became gradually shorter and the last few bursts showed hardly any tails at all. (The tail duration gradually decreased from ~25 s to <10 s). Therefore, a large part of the tails in bursts from these sources must be intrinsic to the source.

(2) Different kinds of bursts are emitted by MXB1730−335 (Rapid Burster)[7]. Besides the Type I bursts, the Rapid Burster also emits Type II bursts, whose spectral evolution is very different in that the softening during burst decay is much less pronounced than in the Type I bursts[7,8]. If the mechanism proposed by Alcock and Hatchett were to explain the tails of the Type I bursts such softening tails should also be observed after these Type II bursts, but they are not.

(3) Furthermore, in spite of the large distance and interstellar reddening[15] of MXB1730–335 a superposition of 12 small Type II bursts by Ulmer *et al.*[16] shows that these bursts do not have tails longer than ~2s. This observation alone shows that the tails caused by interstellar scattering have a duration $\leq$2s for the Rapid Burster.

(4) According to Alcock and Hatchett the optical depth τ_X for scattering of X rays on 3-μm grains has to be small. Hence, the energy observed in the tails of X-ray bursts is proportional to τ_X (see their equation (16)). We make the reasonable assumption that the distribution of the large grains follows that of the small ones (which are responsible for the interstellar reddening). Most of the heavy elements in the interstellar medium are probably locked up in grains[9]. Therefore, according to this model, one would expect a positive correlation between the relative strength of the burst tails and the interstellar absorption. The latter is commonly expressed in terms of the equivalent interstellar column density of hydrogen $N_{\rm H}$. We tested this prediction by comparing the observed values of $N_{\rm H}$, as obtained from fits to the burst spectra[4] with the ratio ε of the energy in the burst tails to the total burst energy. In those cases where optical evidence is available on the interstellar reddening (MXB1659−29; 1735−44 and the galactic centre burst sources)[10–12] good agreement exists with these $N_{\rm H}$ values, using a relationship between $N_{\rm H}$ and E_{B-V} (see refs 13 and 14). It is somewhat ambiguous to determine where the tails start, but this is of no consequence; the range of values of $N_{\rm H}$ is at least a factor of 10, and this is much larger than the uncertainties introduced by the above ambiguity.

There is no correlation between ε and $N_{\rm H}$ for the 10 burst sources studied previously[4]. The values of ε for the sources with $N_{\rm H} \leq 10^{22}$ cm^{-2} range from 0.35 to 0.65. For sources with $N_{\rm H} > 10^{23}$ cm^{-2}, ε ranges from 0.3 to 0.5. These results clearly exclude that the tails are the result of grain scattering, because in that case we would expect values of ε to be approximately proportional to $N_{\rm H}$.

It may be argued that, as dust particles approximately midway between the burst source and the observer dominate the scattering mechanism, this lack of correlation reveals local concentrations of dust around some of the burst sources. In that case the typical value of the 'non local' column density is $\leq 10^{22}$ cm^{-2}, corresponding to interstellar extinction values of $\leq$4 mag. According to Alcock and Hatchett this is insufficient to produce a significant observable effect.

The model of Alcock and Hatchett (although it may work at different time scales and lower intensity levels) does not account for the observed tails of X-ray bursts. These tails are predominantly intrinsic to the X-ray burst mechanism.

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A possible practical application of heavy quark physics

A POSSIBLE method for storing very large amounts of useable energy within small volumes is described here. Our proposal is based on the existence of two new, virtually stable, forms of matter that annihilate each other with enormous energy release, but neither of which annihilates ordinary matter (and can thus be stored easily). The existence of these new forms of matter is suggested[1,2] by the phenomenology of heavy quarks and also by a large class of widely discussed unified field theories.

It has been proposed[1,2] that the b quark, the constituent of the T meson[3-5], might be a colour sextet and as such probably be absolutely stable. The possible absolute stability of the b quark has also been explored from a different viewpoint[6-8]. We assume[1,2,6] that the electric charge of the b quark equals $-\frac{1}{3}$ times that of the proton. The b quark carries colour and is therefore confined inside colourless hadrons, that is, observable baryons and mesons. The lightest baryons containing a b (or $\bar{b}$) quark are then $B^+ = buu$, $B^0 = bud$, and the lightest mesons are $M^+ = \bar{b}u$ and its antiparticle $M^- = b\bar{u}$. Being each other's antiparticles M^- and M^+ have the same mass: $m_{M^+} = m_{M^-}$, while the B^+ and B^0 masses differ: $m_{B^+} \neq m_{B^0}$. For $m_{B^+} < m_{B^0}$ and $|m_{B^+} - m_{M^-}| < m_p + m_{\pi^+} \simeq 1{,}078$ MeV the hadrons B^+ and M^+ are absolutely stable and B^0 will undergo β-decay. Conversely, for $m_{B^0} < m_{B^+}$ and $|m_{B^0} - m_{M^-}| < m_p \simeq 938$ MeV, B^0 and M^+ are absolutely stable. Standard quark model reasoning strongly suggests the validity of these meson–baryon mass-difference inequalities. Thus the meson M^+ and one of the baryons, B^+ or B^0, are expected to be stable. Whichever baryon is stable (B^+ or B^0) the following three observations apply: (1) neither M^+ nor B^+, B^0 contain $\bar{u}$ or $\bar{d}$ antiquarks and therefore do not annihilate ordinary matter; (2) the positively charged hadrons M^+ and B^+ can form hydrogenoid atoms, which we denote $\mathcal{M}(M^+e^-)$ and $\mathcal{B}(B^+e^-)$, with ordinary negative electrons. These electrically neutral atoms are stable and, as they contain electrons rather than positrons, they do not annihilate ordinary matter either. Hence they may participate in ordinary chemical reactions; (3) M^+ contains one $\bar{b}$ but B^+, B^0 each contain one b quark, so that M^+ can annihilate with both B^+ and B^0.

Several intriguing new possibilities arise. The most attractive one is to assume first stable M^+ and B^+. The resulting hydrogenoid gases $\mathcal{M}_2$ and $\mathcal{B}_2$ may be stored in conventional containers and transported separately, or the compound $\mathcal{M}\mathcal{B}$ may be formed and transported itself. Other more complex chemical compounds would certainly exist. These two new forms of matter $\mathcal{M}$ and $\mathcal{B}$ can be induced to annihilate with considerable release of energy, ~10 GeV per reaction, with typical nuclear cross sections.

The case of a stable M^+ and B^0 is more subtle. Perhaps the B^0 can be attached to a conventional nucleus forming an exotic isotope. For example, if a stable heavy deuterium consisting of B^0p^+ exists, then the discussion of the preceding paragraph applies with $\mathcal{B}$ replaced by $B^0p^+e^-$. Other stable or long lived quasi-stable isotopes may exist. If no stable nuclei containing B^0 exist, then this substance would have to be contained by itself, perhaps using a 'magnetic bottle' with a field of non-uniform gradient exploiting the B^0 magnetic moment. In this case there would no no Coulomb barrier to overcome in initiating annihilation reactions.

All these possibilities seem to be within the realm of conventional technology. Stable quarks would, therefore, offer the possibility of storing very high (useable) energies within small volumes. The technological possibilities are self-evident. It is, as we have found, an inexpensive device to store antiprotons.

We emphasise that experimentally both the existence and the stability questions for the B^{0+} and M^+ hadrons are still open. A further problem is to provide sufficient amounts of B^{0+} and M^+ matter in a reasonable time interval. Nevertheless, the prospect of this new energy source is worth pointing out at this time.

Finally, should the b quark not be absolutely stable or sufficiently long lived, heavier quarks may exist which are.. If carrying $-1/3$ or $+2/3$ proton electric charges, any such quark would be as good in the applications envisaged here.

Since we submitted this note two experimental searches[9,10] for stable hadrons in the 4–10 GeV c^{-2} mass range have yielded negative results, thus making the existence of stable b-quark-containing hadrons highly unlikely. One may have to wait for higher quark species. We have also learned of a paper of H. Fritzsch[11], in which the results given in refs 1, 2 are independently obtained.

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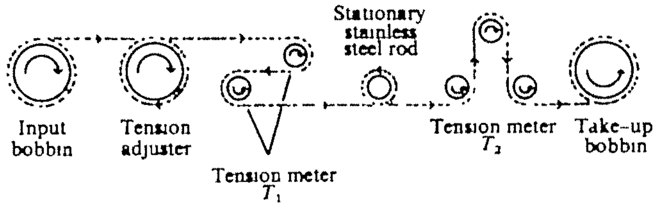
Dyeing increases the friction of synthetic polymer yarns

WE show here that the friction on metal of poly(ethyleneterephthalate) (PET), nylon-6,6 and polyacrylonitrile (PAN) yarns increases when the yarns are dyed, each with a different dye. This phenomenon is well known in the textile industry, but it has only been investigated quantitatively in one case, the friction of dyed wool on metal[1]. Our results show that it is a general phenomenon, and that it is possible to change the mechanical properties of a polymer by the addition of small molecules which have an affinity for the polymer chains.

The PET, nylon-6,6 and PAN yarns were dyed with a disperse, an acid and a basic dye respectively in various conditions (see legends to Figs 2 and 3). The friction of the dyed yarn samples, of the purified but otherwise untreated yarns, and of

Fig. 1 Measurement of yarn friction (see ref 3 pages 189, 210). Dyed and undyed yarn samples were originally wound on bobbins. The yarn passes round a pulley with a ferromagnetic disk at its centre: the input tension of the yarn is adjusted by moving a permanent magnet nearer or further from the disk. Input tension T_1 is measured by a tension meter consisting of two small pulleys one of which is on a spring loaded arm. The yarn passes once round a stationary steel rod 1 cm diameter then over three small pulleys of a second meter: the central pulley is attached to the plates of a condenser, and its capacitance is recorded continuously and calibrated to measure the output tension, T_2. The yarn is wound on to a bobbin at a constant speed of 12.8 m min^{-1}. Pulleys and the rod were cleaned with acetone before each determination



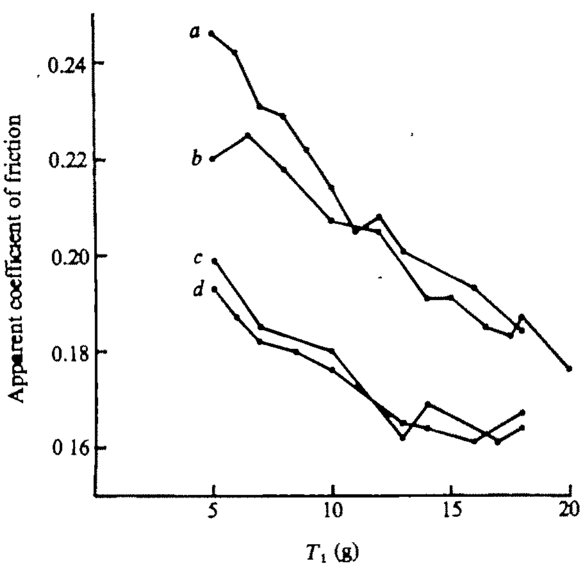


Fig. 2 Decrease in the apparent coefficient of friction of dyed and undyed nylon-6,6 yarn as the input tension is increased: yarn dyed with *a*, 10% and *b*, 7% dye in the dyebath, *c*, undyed yarn, *d*, yarn treated in the same conditions as the dyed yarns but with dye omitted from the bath. Samples of nylon-6,6 yarn (100 m) were purified by soxhlet extraction for 12 h with 60/40 v/v benzene–methanol mixture. The ratio of the dyebath volume to dry weight of the yarn was 30:1. Each yarn sample was immersed in distilled water containing 4% v/wt formic acid for 15 min at 50 °C (all percentages are based on the weight of the yarn). 1% Nylomine Black A-B (ICI), an acid dye, was dissolved in a small volume of the dilute formic acid solution and added to the dyebath, which was raised to 95 °C in 45 min with stirring, and maintained at 95 °C for a further 45 min. The bath containing the yarn was allowed to cool to room temperature. The yarn was rinsed in distilled water and dried at room temperature. Further samples of nylon-6,6 yarn were dyed in the same way in baths containing 2–10% dye (increments of 1%). In the control (*d*), a yarn sample was treated in the same way, except that dye was omitted from the bath. Weight of the yarn, $0.252\ \mathrm{g\ m^{-1}}$, delustred with 0.38% TiO_2.

the yarns treated in the same conditions as the dyed yarns but with the dye omitted from the bath (blank dyebath), were measured using a capstan apparatus (Fig. 1) in which the yarn passes round a stationary stainless steel rod at a constant speed. The apparent coefficient of yarn friction on metal is calculated from the yarn tension T_1 before it passes round the rod and the tension T_2 after the rod.

Assuming that the coefficient of friction of the yarn, μ is related to the frictional force F and the normal force W by:

$$\mu = \frac{F}{W} \tag{1}$$

and assuming that the yarn is inextensible and completely flexible, it can be shown (page 374 ref. 2, page 47 ref. 3) that in the capstan experiment

$$\mu = \frac{1}{2\pi}\ln\left(\frac{T_2}{T_1}\right) \tag{2}$$

It has been shown experimentally that μ determined in this way for a given yarn is not a constant but varies as the input tension T_1 is varied (page 47 ref. 3). μ also depends on the diameter of the rod[3] and on the speed of the yarn[4] This is because equation (1) is not strictly obeyed by polymers sliding on metal, and because yarns are not completely flexible and inextensible[5,6]. The rod used and the speed of the yarn were the same throughout our experiments, and Fig. 2 shows how the apparent coefficient of friction of undyed and dyed nylon-6,6 yarn, calculated from equation (2) alters as T_1 is varied. Dyed and undyed PET and PAN yarns show a similar decrease in the value of μ as T_1 is increased.

The friction of each yarn purified but undyed, treated in a blank dyebath, and treated with various amounts of dye, was compared by calculating the apparent values of μ at two chosen values of T_1. The results (Fig. 3) show that for nylon-6,6, PET and PAN yarns the yarn friction on metal increases as the amount of the appropriate dye in the dyebath is increased (that is, as the amount of dye absorbed by the yarn is increased). The comparison of dyed yarn with two controls (undyed and blank dyebath) suggests that the greater friction of the dyed yarns must be due to the dye absorbed by the yarns and cannot be

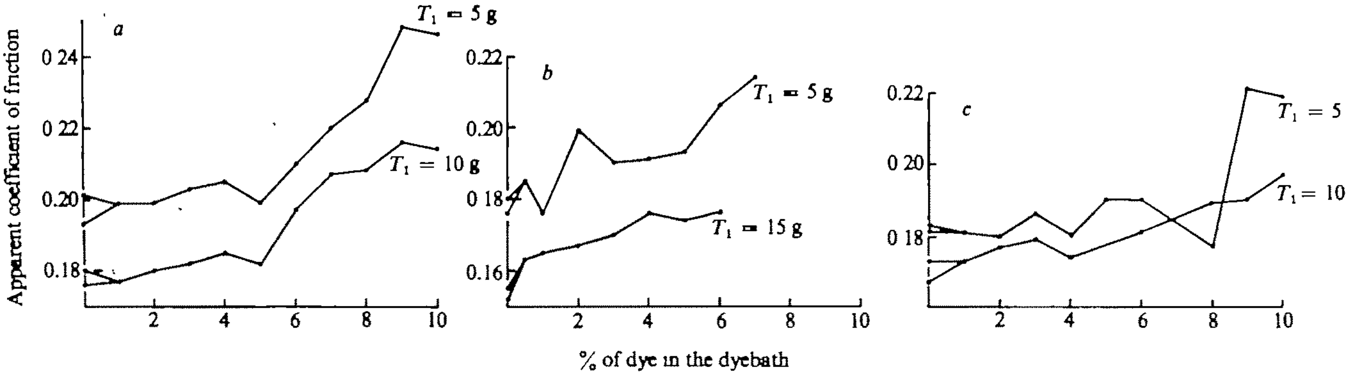


Fig. 3 Increase in the apparent coefficient of friction of nylon-6,6 (*a*), PET (*b*) and PAN (*c*) yarns as the weight of dye in the dyebath is increased. Determinations were made at 5 g and 10 g or 15 g input tension. 100 m samples of all three yarns were purified, dried and weighed as for Fig 2. Nylon-6,6 yarn was dyed as described for Fig. 2. The ratio of the dyebath volume to the yarn weight was 30:1. An aqueous 0.3% solution (v/v based on the volume of water) of Optinol B (Yorkshire Chemical Co.) was adjusted to *p*H 5 with acetic acid and heated to 60 °C A PET yarn sample was placed in it. After 10 min, 0.5% Serilene blue 2G (Yorkshire Chemical) was dissolved in a small volume of the Optinol B solution and added. After stirring for 5 min at 60 °C the temperature was raised slowly to boiling and maintained at the boil for 1 h. The dyebath containing the yarn was allowed to cool to room temperature and the yarn was rinsed in distilled water and dried in air Further yarn samples were dyed in the same way in dyebaths containing 1–7% dye Another yarn sample was treated in the same way in a *p*H 5 Optinol B solution without dye. Weight of the PET yarn, $0.044\ \mathrm{g\ m^{-1}}$; delustred with 0.26% TiO_2 A sample of PAN yarn was wetted with distilled water and put into a dyebath containing 1% acetic acid, 1% sodium acetate, 1% Synacril blue R (ICI) and 1 9% Ataxal LC-RA (ICI) cationic retarder, at 95 °C The dyebath was raised to the boiling point over 10 min. Boiling was continued for 45 min, and then the dyebath containing the yarn was cooled to room temperature and the yarn was removed and rinsed in distilled water. The procedure was repeated using 2% dye and 1.6% retarder, 3% dye and 1 3% retarder, 4% dye and 1.0% retarder, 5% dye and 0.8% retarder, 6% dye and 0.5% retarder, and 7%, 8%, 9% and 10% dye without retarder. A sample of yarn was also treated at the same temperatures for the same times in a bath containing only 1% acetic acid, 1% sodium acetate and 1.9% retarder. Weight of the PAN yarn, $0.124\ \mathrm{g\ m^{-1}}$, delustred with 0.32% TiO_2. For the nylon-6,6 and the PAN yarns the higher value of the apparent coefficient of friction at 0% dye was the value obtained with the purified yarn and the lower value was the value obtained with the yarn treated in a blank dyebath. For the PET yarn the opposite was found.

explained, for example, by the removal of lubricant from the yarns during dyeing, or by deposition of oligomers on the surface of the PET yarn during dyeing.

In the simple adhesion theory of friction, Bowden and Tabor (ref. 2 pages 52, 214) propose that when one material is sliding over another the coefficient of friction μ is equal to s/p where s is the force per unit area necessary to shear the softer material, and p is the pressure at which the softer material ceases to deform elastically and begins to flow plastically (that is, p is the yield pressure of the softer material). Our results imply that in drawn synthetic fibres, dyeing increases the shear strength of the fibres in the direction of the fibre axis more than it increases the yield pressure. This may be a consequence of the preferred orientation of the polymer molecules in the fibre direction. Small molecules, not necessarily coloured, with an affinity for the polymer chains may alter the mechanical properties of a polymer by forming weak, non-covalent crosslinks between the chains.

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Horizontal structure of scintillation-producing irregularities in southern mid-latitudes

THERE is little information on the instantaneous macroscopic structure of scintillation regions affecting radio communications from satellites. The horizontal structure of scintillation regions has been studied using one or two orbiting satellites or a geostationary satellite. In these studies the temporal and/or spatial resolutions of the methods were poor which made it difficult to evaluate the latitudinal and longitudinal distributions of scintillations over a short period. The temporal resolution is important as scintillation patches seem to drift with a median velocity of 100 m s^{-1}, changing their position from one satellite pass to another[1]. To overcome these difficulties I have used all the available VHF transmissions (frequency range: 136–150 MHz) from the following non-synchronous satellites (satellite orbit's inclination is given in brackets): six US Navy Navigational Satellites (90°), NOAA 3 and NOAA4 (102°), Nimbus 4 (98°), Nimbus 5 and Nimbus 6 (100°), Copernicus (35°), Geos 3 (102°) and Kiku (47°). Amplitude transmissions from these satellites (positioned at altitudes: 700–1,500 km) were recorded on a continuous basis during the periods of increased scintillation activity: summer (November–January 1976–77 and 1977–78) and winter (May–July 1977). The recording station was situated at Brisbane (27.5° S and 152.9° E geogr. lat and long, and 37.2° S invar. lat). The definition of scintillation index (SI) has been given elsewhere[2].

On occasions scintillation activity coincided with a relatively large number of satellite passes during a period of one hour or less. It was assumed that the position of scintillation patches did not change greatly from the first to the last recorded pass during this time. Figure 1 shows examples of horizontal extents of scintillation patches recorded for closely spaced satellite passes during summer night-time (*a* and *b*) and winter daytime (*c*).

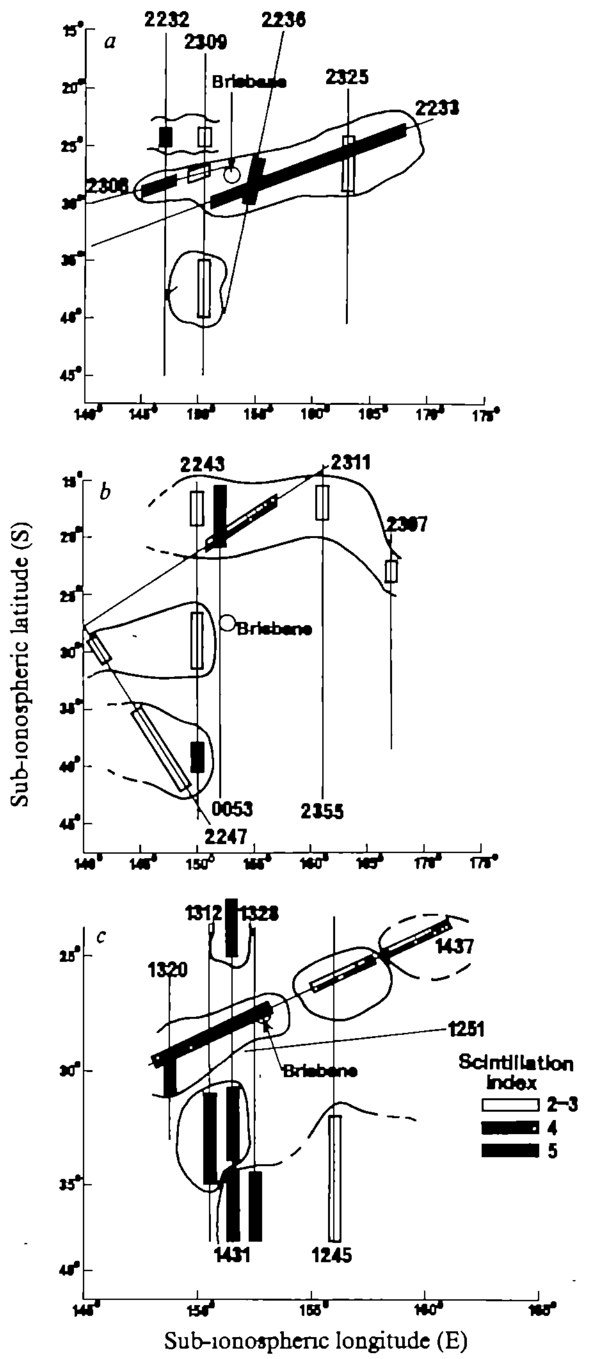

Fig. 1 Examples of sub-ionospheric scintillation regions (rectangulars and curves) derived from amplitude scintillations in the VHF transmissions from orbitting satellites. Thin lines refer to the satellite orbits in the vicinity of the recording station at Brisbane. The numbers indicate the local times (150° EST) of the nearest predicted approaches of satellites. The magnitude of scintillation index (SI) is indicated whenever scintillations are present. *a*, 19 January 1978; $h = 400$ km; *b*, 3–4 January 1977, $h = 400$ km, *c*, 3 July 1977; $h = 100$ km.

Inspection of data from a vertical-incidence ionosonde operating at the same site indicated the presence of intense spread-F and sporadic-E activity coinciding with the periods (*a*, *b*) and (*c*), respectively. From this it was assumed that scintillation-producing irregularities were positioned at altitudes of 400 km (night) and 100 km (day). In Fig 1*a* scintillations in the

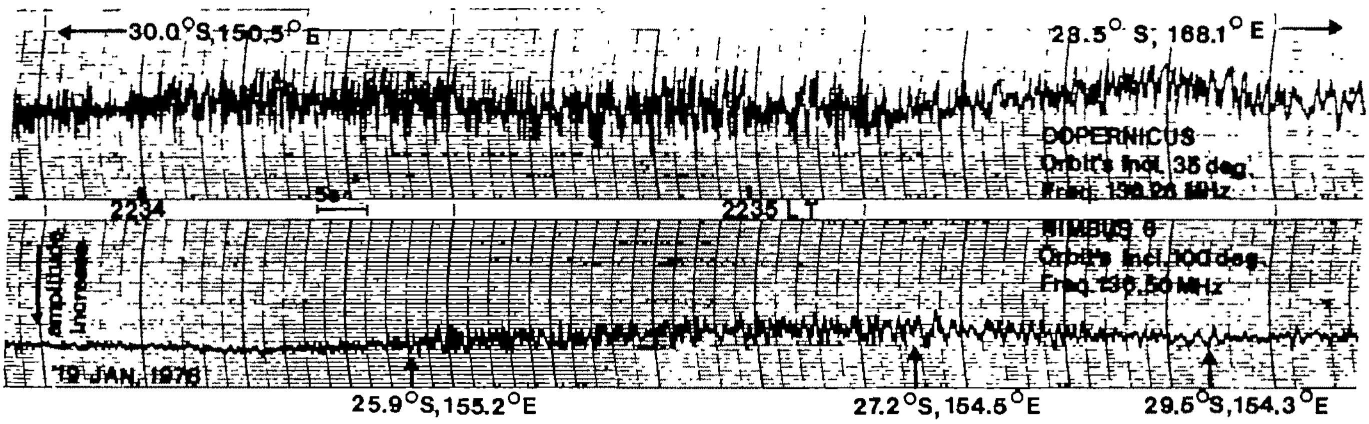

Fig. 2 An example of two simultaneous amplitude recordings of radio-satellite transmissions at frequencies of 136.26 MHz and 136.50 MHz from two orbiting satellites moving mainly in longitude (upper trace) and latitude (lower trace). The sub-ionosphere positions of the limits of scintillation regions are indicated by arrows; the central arrow at 27.2° S and 154.5° E refers to the same sub-ionospheric position for both satellites.

vicinity of Brisbane consisted of an elongated patch extending from about 145° to 170° E longitude. The latitudinal extent was narrower as can be seen from the amplitude data for the north–south passes at 22.36 and 23.25 LT. In particular, two almost simultaneous passes at 22.36 LT (north–south) and 22.33 LT (east–west) revealed a ridge-like formation in scintillations. This can be further seen in Fig. 2 which shows the amplitude scintillation data for these two passes. It is evident that scintillation activity decreased rather rapidly for the north–south pass (Nimbus 6) and continued unbroken for the east–west pass (Copernicus). In addition to the main scintillation ridge two scintillation patches were recorded north and south of the station. The presence of longitudinally extended scintillations is also evident in the second period (Fig. 1*b*) when a sheet-like irregularity was formed north of the station. Two isolated irregular patches were formed west and south–west of the station. Several isolated patches were recorded during daytime in winter (Fig. 1*c*). Once again scintillation causing irregularities had a large longitudinal extent. It is interesting that the irregularity positioned between 150° and 152° E was detected by the east–west pass (14.37 LT) only; no scintillations were recorded in this region for north–south passes at 14.31, 13.28 and 13.12 LT. The phenomenon was not isolated and could also be seen in Fig. 1*a* when scintillation patches recorded for east–west pass at 23.08 LT were not present for two north–south passes at 22.32 and 23.09 LT.

It was possible to obtain 12 records as in Fig. 1 for which at least three satellite passes were recorded with an average time separation of 20 min. In all cases minimum longitudinal extent of scintillations varied from 20° to 30°. Latitudinal extents varied from 3° to 12°, the median value being from 4° to 6°. The horizontal extents of scintillation patches recorded for low orbit's inclination satellites (east–west passes) were several times larger than those for polar orbit's satellites (north–south passes).

Similar results for scintillation occurrences in southern mid-latitudes were also reported from a single polar orbit's satellite transmitting at 20 MHz (ref. 3). It was found that night-time scintillation-producing irregularities occurred in patches with the horizontal extents of 2° to 3° in latitude and more than 15° in longitude. The presence of longitudinally extended irregularity sheets was also reported from a satellite scintillation experiment in Alaska[4]. The ridge-like enhancement in scintillations was associated with the occurrence of the auroral to subauroral transition boundary in the ionospheric total electron content. My results indicate, however, that the sheet-like irregular regions are not limited to a particular spatial position but can occur at various latitudes near the recording station. This might point to a hitherto unrecognised structure of irregularities characterised by a preferential longitudinal extent. A possible link can be established between this type of irregularities and formation of mid-latitude stable auroral red arcs (SAR) which are aligned in longitude with constant L-shell. Their latitudinal extent is limited between 2° and 4° (ref. 5). There is evidence that SAR are associated with ionospheric scintillations in the UHF range of radio-satellite transmissions[6].

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Detection of ^{14}C using a small van de Graaff accelerator

THERE have been several recent reports of the detection of ^{14}C in modern carbon samples using large van de Graaff accelerators[1-3] and cyclotrons[4] as mass spectrometers. The attraction of using such systems is the possibility that very small samples (~5 mg) might be quickly and accurately dated. We have considered the design criteria for a mass spectrometer incorporating a

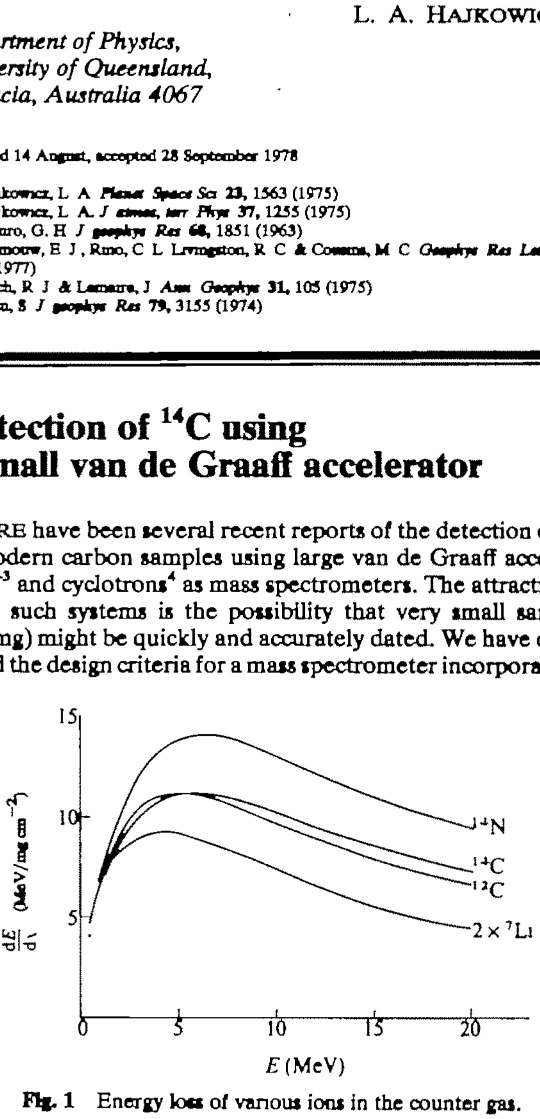

Fig. 1 Energy loss of various ions in the counter gas.

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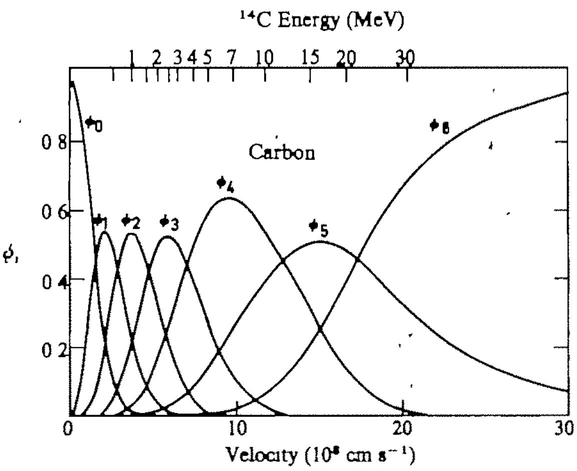

Fig. 2. Charge state fractions for ^{14}C ions after passing through thin carbon foils

tandem van de Graaff accelerator to be built specifically for carbon dating and in this letter we present data which show that the measurements are possible using much smaller and simpler systems than those discussed previously.

The most important system parameter to establish is the required terminal voltage as this will set the size and cost of the facility. The chief advantage of using a high energy mass spectrometer is that ions can be uniquely identified by measuring their energy and specific energy loss. The energy loss depends on the energy, mass and charge of the nucleus as shown in Fig. 1 (ref. 4). It can be seen from Fig. 4 that at low energy this identification becomes extremely difficult. In practice a clean measurement can be made for carbon ions if they have an energy of more than 5 MeV after passing through the dE/dx part of a gridded ionisation detector[5]. If the counter is set to give two dE/dx measurements with about 1 MeV loss in each, and if there is about 1 MeV loss in the counter window and in the poor field region near the window, then the minimum safe energy is 8 MeV. Figure 2 (ref. 6) shows the charge state fractions for ^{14}C ions being stripped in thin carbon foils. It can be seen that at 2.5 MeV the stripping to the 3^+ charge state reaches a maximum so that a satisfactory design would have the tandem terminal at 2.5 MV producing 10 MeV ^{14}C^{3+} ions.

Furthermore recent measurements at Rochester have shown that doubly charged metastable molecular ions of mass 14 exist whose lifetimes are long enough for them to introduce a considerable background. This problem can be avoided by selecting the 3^+ charge state, for which the lifetimes of metastable ions are known to be very short.

We have demonstrated that 2.5 MeV is a suitable choice of operating voltage using the experimental system shown in Fig. 3.

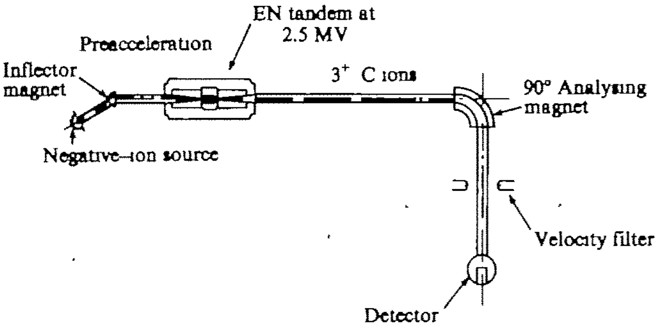

Fig. 3. Beam transport system for the ^{14}C mass spectrometer

The carbon samples were introduced into a reflected beam sputter source[8] in the form of graphite pellets made by pyrolising methane at a pressure of about 500 torr. In this type of ion source a beam of caesium ions, produced by surface ionisation, is accelerated and then focused on to a pellet from which some of the sputtered particles emerge with a negative charge. The intensity of our ^{12}C$^-$ beam was of the order of 5 μA and the intensity of the mass 14 beam was found to be about 3 nA, consisting mainly of ^{12}CH$_2^-$ and ^{13}CH$^-$ and a small fraction of ^{14}C$^-$. For a modern carbon sample, this fraction corresponds to about 37 ^{14}C^{3+} ions per second arriving at the detector. After acceleration and stripping the beam was momentum analysed to select the 3^+, mass 14 beam with a resolution of 1 : 10^3. A Wien filter with a velocity resolution of 5% removed most of the ions which had the correct magnetic rigidity but which suffered charge exchange outside the gas stripper. The resulting ions were detected in a gridded ion chamber filled with isobutane at

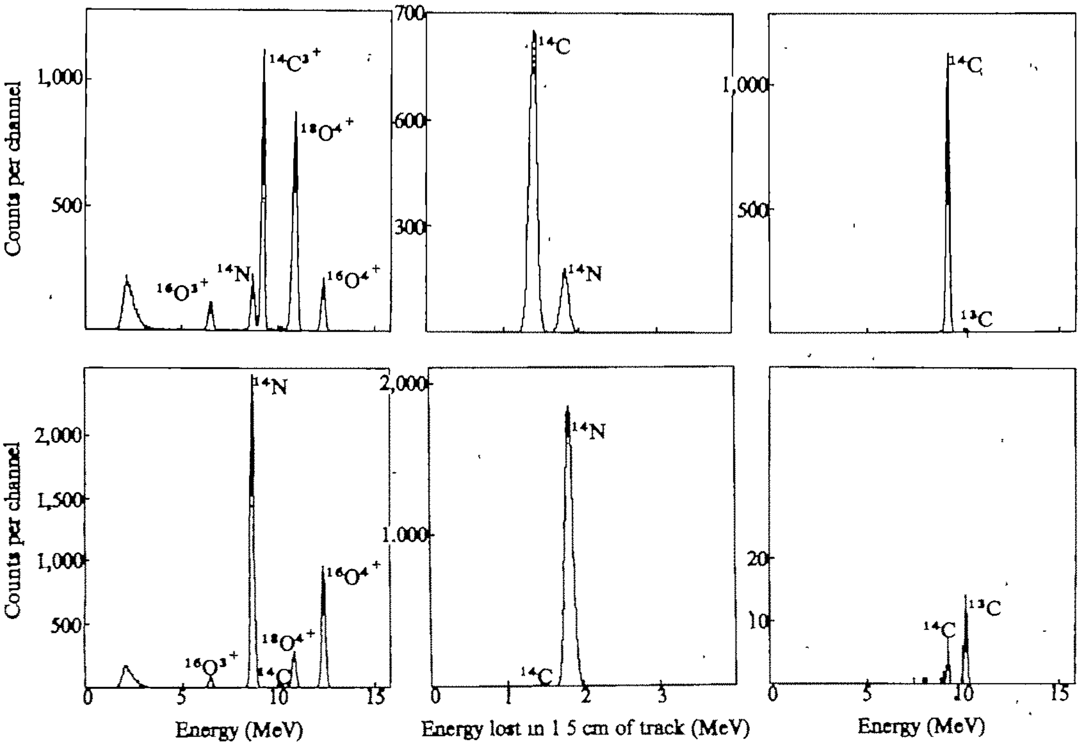

Fig. 4 Energy and energy loss spectra obtained for modern (top line) and old (bottom line) carbon samples. Left, total energy spectrum for carbon; centre, ΔE_2 for ^{14}N+^{14}C; right, total energy spectrum for carbon ions.

20 torr. The anode of this chamber is split into three sections running normal to the ion trajectories so that two measurements of energy loss (dE/dx) could be made in addition to the total energy. For each ion entering the detector, the ionisation measurements were digitised and recorded on magnetic tape using an on-line computer. The data were then processed to produce various spectra which allowed the ^{14}C count to be determined. Further experimental details may be found in ref. 9.

Typical data for modern and old carbon samples are shown in Fig. 4. It is clear from the energy spectra that the energy resolution obtained (150 keV FWHM) is sufficient to separate the carbon isotopes cleanly. The dE/dx resolution is sufficient to separate the ^{14}N group from the ^{14}C. Interestingly, the nitrogen group is quite intense at these energies. It was established by scanning the terminal voltage that this nitrogen comes from injected NH^- ions which charge exchange somewhere in the accelerator tubes. By observing that there was no shift in the dE/dx peak for the ^{14}C groups from old and new samples we could rule out a significant contribution from $^{13}CH^{3+}$ or $^{12}CH_2^{3+}$ molecules in this measurement. The background from misidentified particles was less than one count per day.

Our measurements indicate that errors due to cross contamination of samples in the ion source are less than 0.1%. Improved ion sources can produce over 100 μA of $^{12}C^-$ ions, so that 0.1% counting statistics could be achieved in a few hours. In order to make abundance measurements to this accuracy, however, it will be necessary to monitor closely the source output and the transmission through the accelerator. We propose to achieve this by pulsing the injection system to accelerate the ^{12}C and ^{13}C beams for a few microseconds every millisecond. These beams would be measured in separate ports on the analysing magnet. In addition the ion source will alternate between the unknown sample and a standard. It should then be possible to date accurately small archaeological or environmental samples or the products of laser enrichment as discussed in the previous article.

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Enrichment of ^{14}C and radiocarbon dating

THE very low natural abundance of ^{14}C (10^{-12} times that of ^{12}C) and its exponential decrease in time require exceptionally sensitive methods for its measurement in the dating of archaeological and environmental samples. Present methods count β emission from the nuclear disintegration and so require substantial samples (1–5 g), long count times (1–3 d), and great attention to ensure a low level background. Nevertheless, they have been developed to a point where a reproducibility of 0.025% (one standard deviation) has been attained.[1] Here we describe experiments in which dilute ($^{14}C = 10^{-4} \times {}^{12}C$) samples for CH_2O were enriched by a factor up to 100 by selective photolysis of an individual rotational line in the UV spectrum with a tunable laser. Full details of the experiment will be published elsewhere[2]. This letter summarises the process and develops its relationship to existing and potential carbon dating methods.

Formaldehyde is a convenient molecule for isotopically selective photolysis because it has a well-resolved spectrum in the near UV with a quantum yield for dissociation of near unity. From 350 to 340 nm dissociation is entirely due to $CO+H_2$, whereas from 330 to 290 nm about 30% of the quantum yield is to $H+HCO$ (ref. 3). If the radicals are not scavenged the enrichment can be reduced. Enrichment takes place when the molecule is photolysed at a frequency where the $^{14}CH_2O$ absorption cross section is much greater than that for the other isotopes. Previous work[4] has shown that a tunable laser with modest bandwidth (~20 GHz) could enrich ^{12}C from a mixture of ^{12}C and ^{13}C formaldehyde, and that enrichments of up to 30 could be achieved for some C and O isotopes using ion lasers[5]. Because of the vibrational band envelope structure of the electronic transition in CH_2O it is to be expected that enrichment of a heavier isotope from a mixture will be more difficult, and a necessary preliminary to the enrichment described here was a high resolution survey of the absorption spectra of $^{14}CH_2O$ and $^{12}CH_2O$ throughout most of the spectral region of 290–350 nm.

Absorption spectra were obtained with a tunable, frequency doubled dye laser (either a Hänsch type N_2 pumped laser, or the Chromatix CMX-4) transmitted in two passes through 2–3 m of 33% $^{14}CH_2O$ at about 3 torr, and through 6 m of CH_2O at 10 torr. The laser frequency bandwidth was around 3 GHz ($0.1\ cm^{-1}$). More than 30 $^{14}CH_2O$ lines were noted in which the absorption cross section ratio was measured to be greater than 50, and also where the $^{14}CH_2O$ absorption cross section itself was greater than $5 \times 10^{-20}\ cm^2$. Figure 1 compares high resolution spectra of $^{12}CH_2O$ and $^{14}CH_2O$ where such an enrichment line is to be found (at 331.41 nm). The absorption ratio at the spectral resolution used here is 300 ± 100. The majority of these enrichment lines were in the region 318–340 nm despite the fact that at longer wavelengths the isotope shift is less.

Enrichment was carried out by photolysing a sample of CH_2O containing 0.01% $^{14}CH_2O$ in a 3-m cell with two passes for 1–2 h: laser power was typically 0.2–0.5 mW. The CO so produced, in micromole quantities, was collected by trapping with liquid helium, estimated by gas chromatography, and measured for radioactivity with a gas conductivity meter. The specific activity was compared with that obtained from the CO produced by photolysing an equivalent sample of CH_2O with broad band light (mercury lamp), and the ratio of the two specific activities gave the enrichment factor. Enrichment was performed at 318.59 nm and 326.99 nm. The latter wavelength corresponded to the best projected enrichment value and enrichment in the region of 100 was achieved without any attempt at radical scavenging. Improvements to this figure can be expected through minimising pressure broadening effects and improving the laser bandwidth and frequency stability.

As ^{13}C constitutes 1% of natural C, it will become the predominant isotope at enrichments (with respect to ^{12}C) of over 100. Therefore to increase the proportion of ^{14}C in a sample beyond a factor of 100, enrichment with respect to ^{13}C must also be achieved. While frequencies for the optimum enrichment against one isotope will not coincide with those for the other, a study of the overlap of ^{12}C-, ^{13}C- and ^{14}C-H_2O spectral lines shows that substantial enrichment (>10) against ^{13}C can occur simultaneously with high enrichment (≥ 100) against ^{12}C.

We now assess the relevance of laser enrichment to ^{14}C age determination. Enrichment by thermal diffusion of massive samples is already carried out at several radiocarbon laboratories; its requirements of very large samples (~40 g), long

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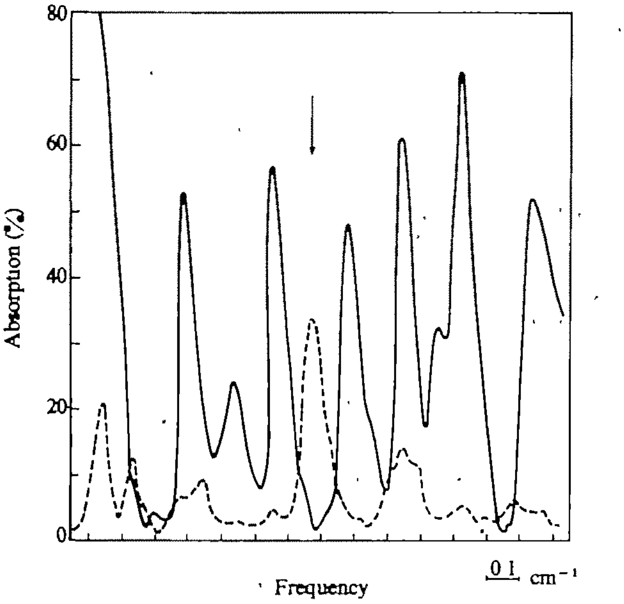

Fig. 1 Comparison of high resolution spectra of $^{12}CH_2O$ and $^{14}CH_2O$. $^{14}CH_2O$ absorption has been reduced by 30 to fit on the same scale.

times (3 weeks) and low enrichment for sufficient product (factor of four) restrict its application to samples too old to date by standard methods (>40,000 yr, <70,000 yr).

First we consider the practical implications for a laser enrichment system; formaldehyde is relatively easy to prepare, either by the catalytic reduction of CO_2 to methanol, followed by catalytic oxidation, or by the mercury photosensitised reaction of H_2 with CO (ref. 5). Its propensity to polymerise requires that the monomer be stored at 77 K, or at low pressure (<20 torr) in apparatus free from adsorbed water. The photolysis product, CO, is easily extracted in distilled form from the photolysis cell.

The major limitation is the laser energy required to enrich macroscopic amounts of sample. A $^{14}CH_2O$ absorption cross section of 10^{-19} cm^2 implies that a photon flux of 0.7 mW cm^{-2} will convert 20% of the $^{14}CH_2O$ per hour. This was confirmed in our photolysis experiments. In a carefully designed system it is clear from the effect of the laser linewidth on the measured isotopic cross section ratio that a bandwidth of less than the Doppler width (of 2 GHz) is desirable. To photolyse 50% of the ^{14}C in a 1 g sample, a laser energy in the region of 100 mW hr is necessary. In general the process is likely to be most useful when extracting 20–50% of the ^{14}C, as the enrichment factor starts to fall once the ^{14}C is significantly depleted. In practice we may expect the required energy to be higher by perhaps a factor of two. Where accurate knowledge of the enrichment factor is needed (for example, in the dating of 'young' samples), simultaneous photolysis of a calibrated standard in a separate cell can be carried out. In this way variations in laser frequency and bandwidth are compensated and do not cause systematic changes in the enrichment factor. The design of the laser will depend on the power expected. It is unfortunate that, despite a careful search, no lines for enriching ^{14}C were found to coincide with the frequencies available from high powered rare gas ion lasers. While it is possible that new lines will be available, the chance of coinciding with an enrichment line is very small. Possible tunable laser systems include Raman shifted tunable rare-gas halide lasers, or dye lasers either frequency doubled following pumping by a high repetition rate Cu vapour laser or a high power cw ion laser, or the direct lasing at 330 nm of p-terphenyl, pumped by a KrF laser.

There are three possible approaches to ^{14}C abundance measurements—counting ^{14}C atoms or ions after mass filtering, counting the nuclear disintegrations and measuring the half life, or integrating the radioactivity over a period of time. Only the second method has been used so far for the routine production of dates.

As 1 g of modern carbon has a disintegration rate of 15 d.p.m. counting methods require samples in the 1–5 g range to be counted for 1–2 d for adequate statistics. The advantage of enriched samples is in permitting much smaller counters to be used, thus reducing the background level (which increases with the counter volume). For a modern sample a minimum of 0.5 g is required to accumulate 1% statistics in 24 hours (with negligible background), and laser enrichment could offer little advantage. (The slight gain in accuracy by reducing the background would be offset by inaccuracies in estimating the enrichment factor). But there is a gain for older samples, provided sufficient material can be processed. Consider the enrichment of a 5 g sample of 38,000 yr age. If 25% of the ^{14}C is extracted, and the typical background of 1 c.p.m. is reduced to 5 counts per hour, in 24 hours of counting the background is estimated to 10% statistics, while the signal is estimated to 4%, and the overall statistical error is about 8%, or 1% of the date. The processing of 5 g samples is rather beyond present laser power capabilities, but it would not be unreasonable to expect these to increase by a factor of five in the next few years.

Integrating the radioactivity, for example in a photographic emulsion or thermoluminescent dosimeter, frees the sample from expensive counting apparatus and allows integration for a long period. In natural samples the dose rate is comparable to the cosmic ray and residual dose backgrounds, but 100-fold enrichment would reduce these to negligible levels[6]. The sample size needed can be very small, and so is much better suited to currently available lasers. For example, 10 mg of carbon enriched to 0.1 mg (from a 40 mg sample) gives a 1 mrad dose in three weeks to a conveniently small quantity of phosphor (Dy:$CaSO_4$), and while thermoluminescent measurements are unlikely to have the accuracy of conventional counting the advantages of this approach in economy and speed are worth pursuing. In principle the same advantages apply to track counting in emulsions[7], where the level of background tracks can be made negligible and therefore do not require sophisticated discrimination.

If all the ^{14}C atoms are detected, 1 mg of modern carbon contains about 4×10^7 atoms. While the degree of mass filtering from ^{12}C and ^{13}C is enormous, mass spectrometric systems could have total efficiences in the region of 5–0.1% so that a requirement for modern carbon of 1 mg samples is reasonable. Such accuracy should make it possible to date many new materials, such as CO_2 in glacier cores, foraminiferas in ocean cores, carbon in archaeological iron and single seeds. The first serious attempt[8] to detect ^{14}C mass spectrometrically was made with a two-stage mass spectrometer of $^{14}C^{15}N^-$. This failed because of high backgrounds from molecular impurities at this mass. The isotope enrichment process described here was originally designed for a novel mass spectrometer system, following an idea of Bennett[9]. Bennett proposed a beam/foil conversion of C^+ at 30 keV to C^-, with mass filtering of both charge states, thus eliminating background due to the presence of ^{14}N (where the probability of forming metastable N^- is extremely low) and molecular species.

Other methods using small samples have demonstrated the advantages of accelerating C^-, stripping to C^{n+}, and accelerating to energies above 10 MeV where their detection signatures can be better identified[10,11]. Another similar proposal is to accelerate C^{2+} in a cyclotron[12]. These methods imply an initial beam current of the order of 1–50 µA, a final count of 10^4 on modern carbon, and a run time of an hour or so. Results with the tandem van de Graaff accelerator show that enrichment is not necessary for the dating of recent samples. This is clearly shown in the accompanying article[13], which presents results of van de Graaff mass spectrometric detection of ^{14}C with an experimental arrangement that would be suitable for the operation of a small facility dedicated to ^{14}C dating. However, it is in extending the age to which samples can be dated that the technique of isotope enrichment will be most important. An enrichment of 100–

300 corresponds to a decrease of 37,000–45,000 yr in age; with the likely limit for direct measurement in the 50,000–100,000 yr region, a very significant extension to dating possibilities can be made. The integrity of the sample may perhaps be the ultimate limit; at 10^5 yr, contamination by 1 p.p.m. of modern carbon will double the ^{14}C content, or make the apparent age 6,000 yr younger. Fortunately with small samples (the minimum sample at this age to give 100 counts with the maximum total detection efficiency of 5% expected in the tandem van de Graaff is 50 mg) it is easier to ensure that the sample has been stringently purified. For example, that extracted collagen from bone has the appropriate (highly distinctive) amino acid decomposition. It has been pointed out[12] that a large error in the abundance determination for samples many half lives old leads to a quite acceptable error in the date, because of their exponential relation, and so 100 counts is more than adequate to estimate a reliable date, provided the background is low enough.

We therefore expect laser enrichment to make a major contribution enabling carbon dating to be reliably applied well into the last interglacial period, once the new mass spectrometric methods have been fully developed. Pre-enrichment on recent samples would permit faster and cheaper radiation dosimetry techniques to be used. In both cases the use of much smaller samples than hitherto possible will bring many new opportunities to archaeological and environmental age measurements.

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Age of the Olby–Laschamp geomagnetic polarity event

SEVERAL excursions or reversals of the Earth's magnetic field throughout the normal polarity Brunhes period which started 690,000 yr ago, have been reported recently; these were summarised by Verosub and Banerjee[1]. The first recent event to be reported was recognised by Bonhommet and Babkine[2] in lavas from the Chaîne des Puys (Massif Central, France) and was named the Olby–Laschamp magnetic event, but no precise date for this event has yet been obtained. An upper limit of 20,000 yr was suggested by K–Ar data[3] and has led to attributing to this event several recently discovered young magnetic events[4]. However, subsequent thermoluminescence and K–Ar data[5,6] indicate ages older than 30,000 yr. To clarify these age relationships I report here my dating of the Olby flow by the ^{230}Th/^{238}U radioactive disequilibrium method.

Table 1 U and Th concentrations and (^{230}Th/^{232}Th), (^{238}U/^{232}Th) activity ratios in Olby minerals

Minerals	^{238}U (p.p m.)	^{232}Th (p.p.m.)	$\frac{^{232}Th}{^{238}U}$	$\left(\frac{^{238}U}{^{232}Th}\right)$	$\left(\frac{^{230}Th}{^{232}Th}\right)$
Total rock TR	2.07	8.36	4.03	0.744	0 780 ±0.010
Pyroxene Px.	0 492	1.97	4.00	0 750	0 791 ±0 019
Plagioclase Pl.	0 523	2 29	4.38	0.685	0.783 ±0.018
Magnetite Mt1 80–23 μm	0 951	2.93	3.08	0 970	0.864 ±0.017
Magnetite Mt2 23–7 μm	1.52	4.01	2.63	1.142	0.904 ±0.017

The ^{230}Th/^{238}U method is well adapted to dating volcanic rocks younger than about 250,000 yr. It was successfully used in obtaining the ages of samples of the Irazu volcano (Costa Rica)[7] and of Etna (Sicily)[8]. The method is based on the following chronological equation:

$$\left(\frac{^{230}\mathrm{Th}}{^{232}\mathrm{Th}}\right)=\left(\frac{^{238}\mathrm{U}}{^{232}\mathrm{Th}}\right)(1-e^{-\lambda t})+\left(\frac{^{230}\mathrm{Th}}{^{232}\mathrm{Th}}\right)_0 e^{-\lambda t}$$

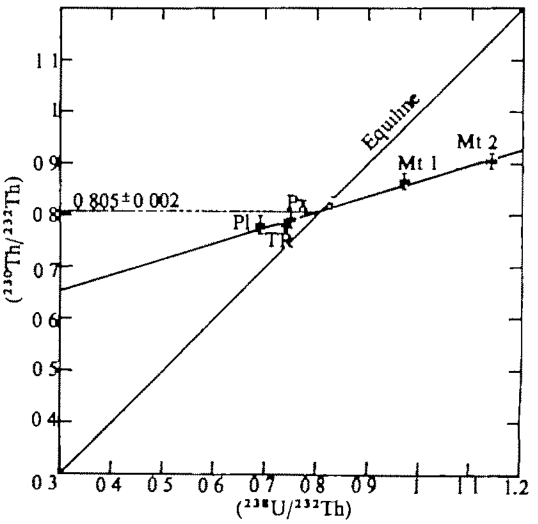
where the ratios are expressed in activity; λ is the ^{230}Th decay constant and ^{232}Th, considered as a stable isotope, is taken as a reference. The equation describes the return towards equilibrium between ^{238}U and ^{230}Th which has a characteristic period $T_{1/2}=75,200$ yr.

Minerals of a volcanic rock crystallise from a magma with different (^{238}U/^{232}Th) ratios due to chemical fractionation between U and Th, but with the same (^{230}Th/^{232}Th)$_0$ initial ratio if isotopic homogeneity is maintained. After a time t has elapsed their (^{230}Th/^{232}Th), (^{238}U/^{232}Th) define a line with slope $(1-e^{-\lambda t})$ from which the crystallisation age may be obtained[9]. The (^{230}Th/^{232}Th)$_0$ initial ratio is given by the intersection between the isochron and the principal bissectrix which is the position of the isochron when equilibrium is reestablished.

Details of the method and the analytical techniques are given elsewhere[7]. U and Th are measured by isotopic dilution and mass spectrometry. Errors in the (^{238}U/^{232}Th) ratios are estimated at 1%. Errors in the (^{230}Th/^{232}Th) ratios arise from counting statistics in α spectrometry measurements (1_σ).

The sample studied is a K-hawaiite with olivine, clinopyroxene, plagioclase and titanomagnetite phenocrysts, from the inversely magnetised flow of Olby[2]. The analyses were carried out on the total rock, clinopyroxene, plagioclase and two fractions of titanomagnetite of different grain size. The analytical results are reported in Table 1 and on Fig. 1. Least-square fitting of the data yield an age of 39,000 ± 6,000 yr and a (^{230}Th/^{232}Th)$_0$

Fig. 1 Internal isochron of the Olby sample, $t=39,000\pm6,000$ yr



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initial ratio of 0.805±0.002. Thermoluminescence dating of quartz pebbles of a palaeosol under the Olby flow yield an age of 41,000±4,000 yr (ref. 10 and G. Valladas personal communication) which agrees well with my data.

Even though the duration of the Olby magnetic event is not known, most of the events found in the period 0–20,000 yr clearly cannot be attributed to the former. Denhan and Cox have shown that if the Olby–Laschamp event had affected the entire Earth's magnetic field it could not have happened between 13,300 and 30,400 yr, or lasted less than 1,700 yr. Other magnetic field disturbances known in the period 30,000–50,000 yr are the Lake Mungo event[12] which lasted from 28,000 to at least 31,000 yr, the Lake Biwa excursion[13] dated by extrapolation at 49,000 yr, an excursion the age of which is estimated at 40,000 yr in sediments from the Indian Ocean[14], and, possibly, an event recently discovered in Iceland (the Maelifell event)[15] which is not dated accurately yet. It is difficult to relate the latter events to the Olby–Laschamp one. If, however, the Lake Mungo and Olby–Laschamp disturbances represent the same event[15] this must have lasted several thousands of years (11,000±6,000 yr). Correlation between young magnetic disturbances, whether local or global, and their interpretation must await accurate dating.

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Phosphatic nodule beds in Victoria and the late Miocene–Pliocene eustatic event

CONTINENTAL evidence of the late Miocene expansion of the Antarctic ice cap and eustatic fall of sea level is still scarce. Here, phosphatic nodule beds in Victoria, Australia and other parts of the world are correlated with these events and with each other. Sedimentological and palaeontological evidence associates the Victorian beds with the late Miocene regressive phase and not with the succeeding early Pliocene phase of deposition. A fauna including whalebone, crabs and the nautiloid *Aturia*, accompanies the phosphatic nodules. The unusual abundance of phosphatic nodule deposits in the late Miocene is attributed to the contemporaneous evaporation, in the Mediterranean, of phosphate-deficient surface water derived from the North Atlantic, which created an enrichment in phosphate in the world ocean, of which the oversaturation surplus was precipitated. A further association of the phosphatic nodule beds with areas of upwelling is postulated to account for their discontinuous occurrence.

Deep-sea evidence indicates that in the late Miocene, the Antarctic ice cap formed and grew to a larger size than the present one, possibly including an extensive partly floating and partly grounded ice shelf[1,2]. Its growth is thought to have caused a eustatic fall in the sea level to at least 55 m below that at present[1]. The late Miocene was the time of maximum regression[1,3] and other events occurring in the higher southern latitudes, including the development of thermohaline circulation, the Antarctic circum-polar current[3], a thermally uniform water mass and the Antarctic Convergence[1,4], all thought to have migrated northwards at the time of maximum glaciation. In the Northern Hemisphere adjacent to the British Isles, a submerged marine terrace indicating erosion by a low sea level in the Miocene has been reported[5,6].

At Hamilton in western Victoria[7], a bed of calcareous clay containing phosphatic nodules rests unconformably on limestone and clay of early and middle Miocene age and contains abundant *remanié* foraminifera from both formations. At Minhamite, also in western Victoria[7], nodules are distributed through sediment of similar type for up to 40 cm above the unconformity, but here the basement and the foraminifera derived from it are of middle Miocene age only. The rich foraminiferal and molluscan faunas at these localities establish the correlation of the beds above the unconformity with the Jemmys Point Formation in Gippsland, the stratotype of the Kalimnan Stage (early Pliocene). *Aturia coxi*, a species known elsewhere from the late Miocene, has been recognised in the nodule bed at Minhamite[8].

At several localities in the Geelong district[9,10], phosphatic nodules occur on or immediately above an erosion surface which truncates marine sediments of early and middle Miocene age. Here they will be considered collectively. The sediment enclosing and overlying the nodules is a fine clay sand, containing a sparse, poorly preserved fauna of Kalimnan age. The Geelong area, alone of all the nodule bed localities considered here, lacks an abundant marine fauna in the formation above the unconformity. Fossil crabs occur frequently in the nodules themselves[10,11].

At Beaumaris, near Melbourne, a phosphatic nodule bed rests unconformably on middle Miocene clay and limestone and is overlain by 6 m of marine calcareous siltstones, above which another lithological and formational change occurs[7,12]. As at Hamilton, the beds immediately above the basal unconformity contain *remanié* foraminifera of both early and middle Miocene age and also *remanié* megafossils[13]. Specimens of *Aturia coxi*, whose matrix suggests that they occur in the interval 0–40 cm above the unconformity, suggest that these lowermost beds are of late Miocene age[8]. Bivalve and gastropod faunas obtained mainly from the beds 0.4–6 m above the unconformity have been shown to have closest affinities with faunas from Kalimnan (early Pliocene) strata and not with those of Miocene age[14,15].

The recent discovery of a phosphatic nodule bed immediately below the Jemmys Point Formation in the Lake Tyers–Lakes Entrance area of Gippsland extends the known nodule bed occurrences a further 330 km eastwards and shows that the nodule bed horizon underlies the Kalimnan stratotype[16]. *Globorotalia puncticulata* occurs almost immediately above the nodule bed in this area. Although this species has been reported from the late Messinian in the Mediterranean area and elsewhere[17], pre-Pliocene records are questionable[18] and the species is not known below the Pliocene in New Zealand[18,19].

One concludes that these Victorian occurrences of the unconformable nodule bed represent an eroded sea floor at the time of the lowest retreat of sea level during the late Miocene regression and is the surface over the rising sea transgressed during the latest Miocene or earliest Pliocene. If the extinction of *Aturia* is truly a Miocene event and the erosion surface represents the lowest still-stand, this apparently occurred slightly before the end of the Miocene.

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At each of the Victorian nodule bed localities referred to here, except Geelong, cetacean vertebrae, ribs, ear bones, teeth and other bone fragments occur more commonly than at any other locality in the Tertiary of Victoria. Where the unconformity below the nodule bed has eroded early Miocene deposits, sharks' teeth also occur in the nodule beds with moderate frequency. Chiu[20] has reported a bed of frequently occurring whalebones, together with sharks' teeth and crabs in Taiwan at a level stated to be exactly on the Miocene–Pliocene boundary. Whether the abundance of whalebone at this time in Victoria, Taiwan and off southern California[21] (see below) is coincidental or indicates a peak in the abundance of whales at this time, is uncertain. Diastems at other levels in the Tertiary of Victoria do not concentrate whalebone.

Note that other well-known phosphorite occurrences, those on the South African continental shelf[22,23]; Chatham Rise[24,25] and Campbell Plateau[26] (east and south of New Zealand respectively); the phosphatic siderite deposits on the eastern Australian continental shelf[27] and those on the continental borderland of southern California[21,28] have also been attributed to a process of 'phosphatisation of late Miocene sediments' (*auct.*). Important associates of the nodules at the off-shore localities of South Africa are *Aturia* sp. and whalebone[22]; of southern California, whale and other marine mammal bones[21] and on the continental shelf of eastern Australia, crabs occur in the nodules as they do in the Geelong district.

The formation of the nodules in the Geelong district was considered by Coulson[9] to have been followed by a time of complete emergence from the sea, yet before deposition of the Kalimnan sands. Although Bowler[10] did not support complete emergence, he agreed that the nodules formed entirely during the regressive phase. The nodules of Chatham Rise are also thought to have been completely emerged after their formation[25] and as the present water depth is about 500 m, it seems unlikely that this occurred as recently as the Pleistocene. Dating of the nodule beds at Beaumaris and Minhamite as late Miocene by the presence of *Aturia coxi*[8], assigns them to the regressive phase and not to the early Pliocene transgressive phase. Evidence from other sources also dates the regression as late Miocene[29,30].

In South Australia, extending 300 km westwards of Hamilton, there are thin Pliocene deposits, usually resting disconformably or unconformably on older Tertiary or even pre-Tertiary rocks[31,32]. Some of them are dated as post-early Pliocene, but collectively they represent the deposits of a transgressive phase suggestive of a eustatic rise in sea level and their faunas contain many genera of foraminifera and molluscs restricted to considerably lower latitudes at present[15]. The Wright Valley in Antarctica was glacially eroded and vacated by the ice before 4.2 Myr BP and subsequently invaded by the sea during the Pliocene[33]. These observations are in accord with the raised southern ocean temperatures estimated for the early Pliocene[34] and apparently accompanying a eustatic rise in sea level.

A temporary northwards migration of the Antarctic convergence during the late Miocene glacial period could result in upwelling of Antarctic bottom water and consequent phosphate deposition on the Australian southern and eastern continental shelves, the Aghulas Bank, the Campbell Plateau and Chatham Rise. There seems little doubt that the upwelling of deep ocean water and phosphate deposition are closely associated[35].

In the Northern Hemisphere, circumstances which led to the formation of the Messinian evaporite deposits in the Mediterranean Basin[36,37] provide a possible mechanism for increasing the amount of phosphate available for precipitation from the world ocean at that time. These evaporites formed from seawater entering the Mediterranean over shallow sills[36] and it has been estimated that they represent 6% of the total dissolved salts in the world ocean at that time[38]. The supply of this evaporite material would have been drawn selectively from North Atlantic surface water, in which the phosphate content is less than in bottom and intermediate water in the Atlantic and other oceans[39]. The result would be a net increase in dissolved phosphate in deep water in all oceans, thus creating a situation in which increased phosphate precipitation in areas of upwelling could be expected to have occurred in the late Miocene.

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Confirmation of ^{137}Cs dating by algal stratigraphy in Rostherne Mere

THE deep sediments of Rostherne Mere, Cheshire (National Grid Reference SJ 745843) offer a promising situation in which to study the relationship between biological stratification and radionuclide dating in the absence of gross bioturbation. The mere is small (46.5 hectare), but relatively deep (30 m maximum), and is reported to be largely devoid of benthic fauna in the persistently deoxygenated deep sediments[1]. The sediments of Rostherne Mere contain the remains of a great variety of algae, in addition to diatoms. The preservation of non-siliceous algae is uncommon, although found in the sapropel deposits of the USSR[2]. In Rostherne the empty spores of *Ceratium hirundinella* O.F. Mull., *Anabaena* species and *Aphanizomenon flos-aquae* (L) Ralfs are recognisable, as are colonies of *Microcystis* species and cells of *Staurastrum* species. The phytoplankton of Rostherne Mere has been recorded at intervals since 1912 (refs 3–5) and sampled more frequently from 1962 (refs 6–8). The recent records (Table 1) show fluctuations in summer dominance, mainly between *Ceratium hirundinella* and *Microcystis aeruginosa* Kütz. emend., but blue–green algae

Table 1 Collected phytoplankton data 1962–77

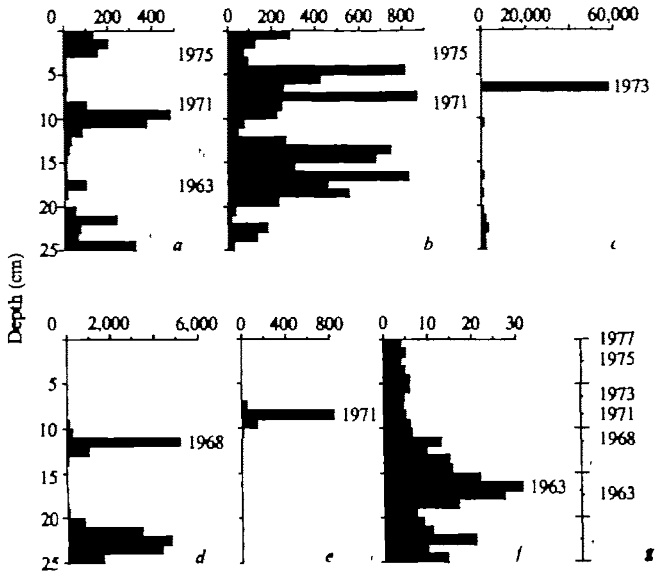
Year	*C. hirundinella*	*Microcystis* spp.	*S. astraea*	*M. granulata*	*C. pseudostelligera*
1977	Rare	Abundant	Rare	Rare	Rare
1976	No record	—	—	—	—
1975	Abundant	Rare	No record	No record	No record
1974	No record	—	—	—	—
1973	Common	Abundant	Abundant	Common	Rare
1972	Common	Abundant	Common	Common	Rare
1971	Abundant	Rare	Rare	Rare	Common
1970	No record	—	—	—	—
1969	No record	—	—	—	—
1968	Rare	Rare	Rare	Abundant	Rare
1967	Common	Common	Common	Rare	Rare
1966	Rare	Abundant	Common	Common	Rare
1965	Common	Abundant	Common	Common	Rare
1964	Common	Abundant	Common	Common	Rare
1963	Abundant	Abundant	Rare	Rare	Rare
1962	Rare	Abundant	Rare	Rare	Rare

Sources: 1962–63, ref. 6; 1964–66, ref 7; 1967–77, ref. 8. No data available for 1976, 74, 70 and 69.

have become increasingly dominant[9]. These changes, combined with occasional maxima of the diatoms *Melosira granulata* (Ehr.) Ralfs, *Cyclotella pseudostelligera* Hust. and *Stephanodiscus astraea* (Ehr.) Grun., can be correlated with algal assemblages in the sediments and permit these to be accurately dated. We now report that such algal remains from the lake sediment have been used to establish a detailed chronology which is shown to confirm the ^{137}Cs dating method.

Three 1-m cores[10] extracted from the deepest area of the mere were examined throughout for algal remains, and all showed the same well-defined stratigraphy. The algal species which are most numerous in the upper 25 cm of the cores are shown in Fig. 1 (*a–e*), and the patterns found reflect the changes in plankton dominance. Thus an 'algal chronology' can be constructed by comparing the sediment biostratigraphy with the observed and dated records of the plankton (Fig. 1*g*). The fact that the remains of many different algae are recognisable in the bottom deposits of Rostherne Mere permits a detailed correlation. Hence, the *Ceratium*-dominant years of 1963, 1971 and 1975 are allocated to the 17-cm, 9-cm and 3-cm horizons, respectively (Fig. 1 *e,d*).

Fig. 1 Algal and caesium stratigraphy. *a*, *Ceratium hirundinella* spores; *b*, *Microcystis* spp. colonies, *c*, *Stephanodiscus astraea* cells; *d*, *Melosira granulata* cells; *e*, *Cyclotella pseudostelligera* cells; *f*, caesium-137 pCi per section; *g*, algal chronology. *a–c*, Numbers expressed per 5 µl of fresh sediment, *d,e*, per 0.1 µl of fresh sediment. A 1-cm thick section of a core contains 29 ml of fresh sediment.



Two of the cores were dated by the distribution pattern of ^{137}Cs (refs 11,12). Sections 1 cm thick were dried and analysed by γ-ray spectrometry[13] using a germanium (lithium) detector. The position of greatest concentration of ^{137}Cs, corresponding to 1963, occurred at a depth of ~17 cm in both cores and this demonstrates consistency between the cores and also agrees with the chronology established from the algal record. The ^{137}Cs profile from one of the cores is shown in Fig. 1 *f*. An earlier core dated by the ^{137}Cs method and reported by Gaskell and Eglinton[14] showed about half the accumulation rate of the present cores. Such variations in accumulation rate at different locations are not unusual and have been discussed elsewhere[15].

The deep sediments of Rostherne Mere have proved to be an ideal environment for the preservation of a variety of non-siliceous algae. Much of the sediment volume is derived from the high algal productivity of the mere, and these autochthonous deposits give a detailed insight into past phytoplankton communities. Also, the high rate of sediment accumulation (~1 cm yr^{-1}) ensures that a 1-cm slice provides adequate resolution of annual increments. The apparent absence of large benthic animals ensures that the ordered structure of the stratigraphic column is not likely to be grossly disrupted by bioturbation, and this is confirmed by the identical position of the ^{137}Cs peak in both the cores analysed.

Such conditions will not necessarily apply in other lakes, where there may be greater diffusion in the sediment column or more significant contribution from the catchment. Rostherne Mere is unusual because of the high autochthonous contribution to the sediment and small inflow of fine particulate allochthonous material. This is the first site in Great Britain at which independent evidence has been found for the validity of the dating of lake sediments provided by ^{137}Cs.

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Temporal decline in attractiveness of honeybee queen tracks

A BODY or surface (such as that of a worker bee or a cage) that has been in contact with a honeybee queen becomes attractive to worker bees[1–3] due to contamination with queen pheromones[3,4]. Little information is available about the durability of those pheromones, apart from Butler's report that 30 min after removal of the queen a cage becomes less attractive to the workers, and loses its attractiveness entirely after a further hour[1]. I report here experimental evidence of a temporal decline in the attractiveness of tracks left on a waxed surface by a queen.

I monitored the accumulation of worker bees on a wire-gause cage which contained a queen[4,5] or had been marked with her tracks on the inner surface. To achieve experimental conditions as near as possible to those inside a hive, the accumulation of bees was determined by weighing the cage with them on it. The cage was suspended from one of the arms of a balance and was let down, through a hole in the ceiling of the hive, over the brood chamber of the test colony. It was possible to put the queen into the cage and remove her from it without taking the cage from the hole. The cage was left over the brood chamber during the test, and the worker bees had easy access to it.

The cages used were covered with wax, and before the experiments they were kept in the hive of the test colony, which had a mated laying queen.

The bees that gathered on the cage containing a live mated queen were weighed. Then the queen was removed and weighing was repeated at 1-min intervals. The data obtained were used to give the relative attractiveness of queen tracks as a function of time (Fig. 1): it demonstrates a clear decline.

My results show that queen tracks can contain at least one pheromone that loses its effectiveness quite quickly, whereas the best known pure pheromone (9-oxodecenoic acid) has been reported[1] to remain detectably active in very small quantities for 7 yr.

The decline of attractiveness may occur either through volatilisation of the pheromone or in some other way. If queen tracks are assumed to form a thin film over a surface, the pheromone molecules in the film being dispersed far enough one from another (and consequently having an equal chance to leave the surface), the density of the molecules in the air near the surface (due to volatilisation) can be shown[6] to change by the factor $\exp(-t/\tau)$ where t is time and τ is a positive parameter independent of t. The attractiveness of queen tracks can be expected to change in the same way. As Fig. 1 shows, the experimental data agree with this. Therefore the temporal change in attractiveness of queen tracks to worker bees is determined by τ ($27.9 \leq \tau \leq 29.2$ min for 95% confidence level). The halflife is 19.3–20.3 min.

Some evidence for a similar rate of loss of attractiveness of queen tracks is provided by Velthuis's observations of interactions between a group of queenless worker bees and "substitute queens" (workers bearing queen pheromones on their bodies). The attractiveness of the substitute queens lasted 5–45 min, the mean duration being 15 min (ref. 3).

Thus queen tracks are attractive to workers (and therefore are perceived by them) for sufficient time to have a possible influence on the behaviour of the workers and the state of the colony. On the other hand, the tracks lose their attractiveness (and cease to be perceived) sufficiently quickly to account for the rapid onset of queenless behaviour in a colony deprived of its queen.

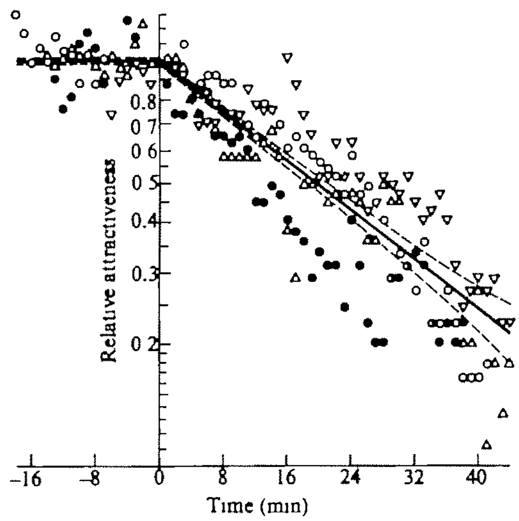

Fig. 1 Relative attractiveness to worker bees of a caged live mated queen (time < 0) and queen tracks left on the inner surface of the cage (time > 0) The different symbols correspond to data collected in four separate tests.

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The living coelacanth *Latimeria chalumnae* does not have a cloaca

POSSESSION of a cloaca is presumed to be a primitive vertebrate character that has been lost independently four times—in lampreys, chimaeras, actinopterygians and eutherian mammals—as the adult morphology of the urinary-genital-rectal openings is different in each group. Hagfishes, sharks, lungfishes, amphibians, reptiles, birds, monotremes, marsupials and *Aplodontia rufa* all possess a cloaca[1]. Millot and Anthony[2] stated that the male *Latimeria* has a true cloaca whereas the female has a rectal opening separate from a common urinary-genital opening. We report here evidence that neither male nor female *Latimeria chalumnae* has a cloaca.

Male and female specimens of *Latimeria* were dissected to follow the digestive, urinary and reproductive tracts to their posterior openings. A plastic strip was inserted into the urethral

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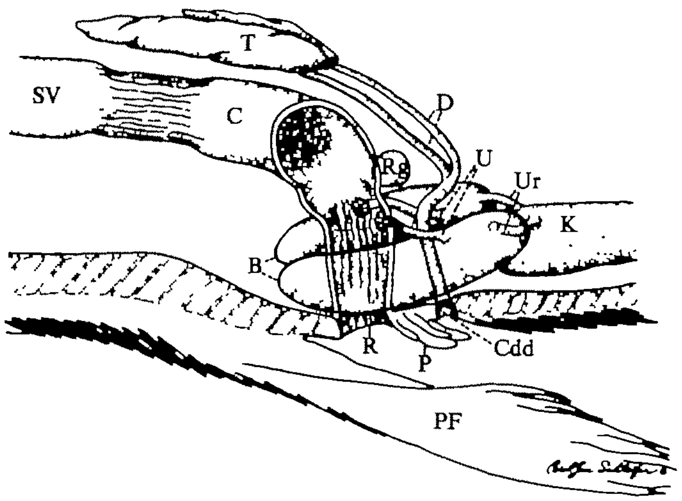
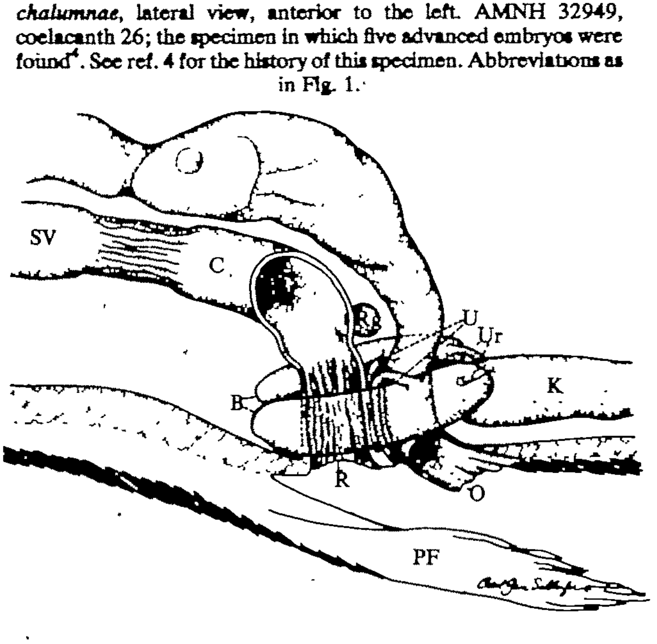


Fig. 1 Male rectal-urinary-genital anatomy of *Latimeria chalumnae*, lateral views, anterior to the left. Based on: AMNH 36941 (American Museum of Natural History) coelacanth 25 in the list of captured specimens[3], 130 cm in total length, captured off Anjouan, Comoro Islands 10 October 1961, obtained from the Muséum National d'Histoire Naturelle, Paris; CAS 24862 (California Academy of Sciences) coelacanth 59, 122 cm in total length, captured off Itsoundzou, Grande Comore, Comoro Islands 1 January 1970; and LACM 6824-1 (Los Angeles County Museum of Natural History), 121 cm in total length, captured in the Comoro Islands in 1970 and not included in the list of coelacanth captures[3]. B, urinary bladder; C, colon; Cdd, common ductus deferens; D, ductus deferens; K, kidney; O, oviduct; P, papillary bodies; PF, pelvic fin, R, rectum; Rg, rectal gland; SV, spiral valve of the intestine; T, testis; U, urethra; Ur, ureter. (In using the above anatomical nomenclature we realise some would question the possibly implied homology between some of the osteichthyan and tetrapod structures. We do not necessarily imply such homology, but merely use the standard terms to name the structures.)

aperture in one of the urinary bladders and pushed through the urethra until it emerged from its distal opening. The other urethra in each specimen was slit open to follow the route of the urethra to the exterior. The oviduct of the female (AMNH 32949) had been opened previously, and was greatly enlarged due to the five advanced embryos it had contained. In the male (AMNH 36941), the left and right ducti deferentes were followed from the testes (the left testis was considerably larger than the right) to the point at which they anastomose, from whence a probe was passed down the common ductus deferens to its external opening. The lower colon and upper rectum were slit open in the male; the entire length of the lower digestive tract of the female had been opened previously.

Fig. 2 Female rectal-urinary-genital anatomy of *Latimeria chalumnae*, lateral view, anterior to the left. AMNH 32949, coelacanth 26; the specimen in which five advanced embryos were found[4]. See ref. 4 for the history of this specimen. Abbreviations as in Fig. 1.



In the male, the urethras descend from the right and left urinary bladder and empty into the posterior wall of the rectum. A series of papillae surround the urethral openings where they join the rectum. The right and left deferent ducts descend from their respective testes and fuse into a common ductus deferens. From the point of fusion, the common ductus deferens descends ventrally and opens externally (Fig. 1). Two additional male coelacanths (CAS 24862 and LACM 6824-1) also have the urethras opening high into the rectum without fusing with each other or with the two deferent ducts. In the female, the right and left urethras descend from the right and left urinary bladders and open directly to the outside on their respective antero-lateral sides of the oviduct. The oviduct itself opens directly to the exterior (Figs 2, 3).

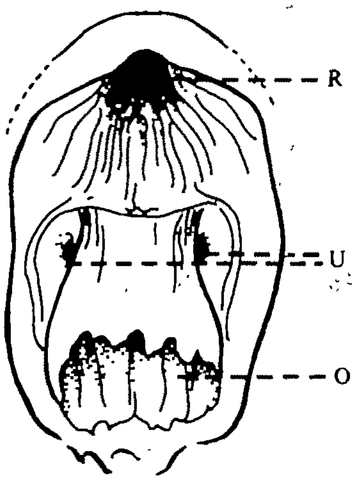


Fig. 3 Ventral view of rectal-urinary-genital openings of female *Latimeria chalumnae*, AMNH 32949. Anterior is to the top, abbreviations as in Fig. 1.

We conclude that neither male nor female *Latimeria* has a cloaca and that the sexes are dimorphic: in the male the urethras empty into the rectum whereas in the female they open directly to the exterior. The anatomy of these openings in the female *Latimeria* is identical with that in many actinopterygians. However, the apparently plesiomorph condition for actinopterygians occurs in *Acipenser*, *Lepisosteus* and *Amia*, namely a rectal opening separate from a common uro-genital opening[1]. This differs from the presumed plesiomorph condition for *Latimeria* which is maintained in the male as a common uro-rectal opening separate from the genital opening. We conclude that *Latimeria* exhibits another independent loss of the primitive cloaca.

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Morphological variance and enzyme heterozygosity in the monarch butterfly

It was shown several decades ago that the reduction in genetic heterozygosity produced by inbreeding in domesticated plants and animals is accompanied by an increase in morphological variability[1,2]. While studies of laboratory populations and results of plant and animal breeding have been instrumental in identifying the relationship between genetic heterozygosity and morphological variability, such investigations often involved substantial changes in genetic heterozygosity, such as are produced when entire chromosomes are made homozygous. For this reason their relevance to the levels of heterozygosity in natural populations is unclear. Until recently attempts to examine this relationship in natural populations were hindered by the inability to routinely estimate levels of genetic heterozygosity in nature. However, determinations of genetic heterozygosity at specific enzyme-loci can now be made by electrophoresis. Recently Mitton[3] found that heterozygotes for a set of five enzyme-loci had reduced multivariate variances for a set of morphological characters when compared to homozygotes at the same loci in populations of the killifish, *Fundulus heteroclitus*. It is very important to determine the generality of this observation for other species populations. I have now examined the relationship between genetic heterozygosity, as identified by electrophoresis, and the variance of morphological characters in the monarch butterfly, *Danaus plexippus*. Study of six polymorphic enzyme-loci in the monarch shows that once again heterozygotes have smaller variances for morphological characters when compared to homozygotes at the same locus.

The data for this study were gathered from over 1,400 monarch butterflies collected in the eastern USA and Canada during the summers of 1974–75. The sample sites, dates of collection, sample sizes, and electrophoretic methods are given in ref. 4. Six polymorphic enzymes were examined; glucose phosphate isomerase(GPI), malate dehydrogenase(MDH), glutamate-oxaloacetate transaminase(GOT), mannose phosphate isomerase(MPI), phosphoglucomutase(PGM), and a TPN-dependent 'nothing' dehydrogenase(NDH). Electrophoretic variation at all six loci is inherited in a mendelian fashion, and it is known that the GPI, PGM and NDH loci are chromosomally unlinked. The chance of linkage between the other pair combinations is small.

Right forewing length and the diameter of the major forewing spot (divided by the length of the adjacent wing vein) were measured on each individual. These characters are normally distributed with a slight skew towards the smaller values. The correlation between characters is statistically nonsignificant ($r = 0.096$, $n = 54$). The characters are sexually dimorphic, and therefore males and females were treated separately in the analysis. There is very little geographic (sample) variation for either character; less than 2% of the total variance in either character can be attributed to between sample differences. Sample character variances are statistically homogeneous.

The association between genetic heterozygosity and morphological variability was examined by comparing the character variances of homozygotes and heterozygotes at each locus. Since there are two independent characters, six enzyme-loci, and both males and females to consider, this results in 24 statistical tests of the null hypothesis of no difference in character variances between homozygotes and heterozygotes. The results of those tests are given in Table 1. Because of the considerable differences in heterozygosity among the loci, and the smaller sample size of females, there is a substantial range of statistical power from test to test; the tests for GOT and MDH in females are the weakest.

The results show that homozygotes generally have larger variances for these morphological characters compared to heterozygotes at the same locus. The results are the strongest for character 2. Overall, 19 of the 24 comparisons show homozygotes with larger variances, and five tests are statistically significant at the 0.05 level. None of the exceptions approach statistical significance. The distribution of test probabilities is noticeably skewed; 14 of 24 tests have probabilities less than 0.20. The results clearly support the hypothesis that heterozygotes at these loci tend to be found near the centre of the morphological character distributions.

Table 1 Comparisons of morphological variances for enzyme homozygotes and heterozygotes in *Danaus plexippus*

Enzyme	Homozygote s^2	Heterozygote s^2	F_s	P
Character 1 (forewing length)				
		Males		
PGM	3.35(371)	3.35(535)	0.97	0.639
MPI	3.68(426)	2.76(306)	1.33	0.004
NDH	3.09(730)	3.29(180)	0.94	0.710
GPI	3.35(492)	2.83(269)	1.16	0.081
GOT	3.16(877)	2.56(63)	1.23	0.110
MDH	3.16(866)	2.56(65)	1.23	0.110
		Females		
PGM	4.07(153)	3.35(229)	1.21	0.091
MPI	3 71(209)	3.53(174)	1.04	0.388
NDH	3.64(310)	3.54(72)	1.04	0.450
GPI	3.85(203)	3.39(170)	1.13	0.193
GOT	3.68(364)	2.50(20)	1.47	0.150
MDH	3.63(354)	3.93(28)	0.92	0.650
Character 2 (forewing spot)				
		Males		
PGM	0.033(382)	0.026(540)	1.27	0.002
MPI	0.030(471)	0.029(300)	1.03	0.406
NDH	0.029(734)	0.033(184)	0.88	0.847
GPI	0.031(501)	0.029(270)	1.07	0.265
GOT	0.033(877)	0.023(63)	1.44	0.050
MDH	0.028(866)	0.026(65)	1.09	0.323
		Females		
PGM	0 033(165)	0.024(229)	1.38	0.021
MPI	0.032(222)	0.022(172)	1.46	0.005
NDH	0.029(319)	0.023(76)	1.26	0.124
GPI	0.030(217)	0.025(178)	1.20	0.084
GOT	0.028(374)	0.030(20)	0.93	0.631
MDH	0.029(366)	0.020(28)	1.45	0.110

Sample sizes are given in parentheses. F_s is the ratio homozygote s^2/heterozygote s^2. P is the probability associated with the one-tailed F test of the null hypothesis; σ^2homozygotes = σ^2heterozygotes. Forewing length variance is in mm^2, and the forewing spot variance is dimensionless. Significant values underlined.

It should be noted that some electrophoretic variants do not represent homogeneous allelic products, but instead are mixtures of different variants with similar electrophoretic appearance[5–8]. Consequently some heterozygotes will be misclassified as homozygotes, and this should tend to obscure the proposed association. The magnitude of this effect cannot be assessed in this study.

Problems certainly arise when assuming morphological variability to be a reasonable measure of developmental homeostasis. Morphological variability may predominantly reflect the segregation of additive and nonadditive genes rather than the expression of developmental instability. An association between enzyme heterozygosity and morphological variability could then be generated by electrophoretic variant alleles being in linkage disequilibrium with the genetic factors determining the morphological variance. It would be expected for electrophoretic heterozygotes to exhibit intermediate morphological values and smaller variances on the average. If this were the source of the observed association, then specific homozygous and heterozygous phenotypes should possess different mean values for the morphological characters. This was not the case. Of the 24 ANOVA tests performed to test for differences in

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means between specific electrophoretic phenotypes, *none* were statistically significant. The ANOVA assumes equality of variances, a condition not satisfied here, however the test is robust to small deviations in homoscedasticity[9]. The outcome of these tests indicates that enzyme homozygosity affects the deviation from the morphological mean, and that the direction of that deviation does not depend on specific homozygous phenotypes.

The connection between genetic heterozygosity and developmental homeostasis led early investigators to propose that developmental buffering was enhanced by enzyme polymorphism along biochemical pathways[10-12], although few methods were available to assess levels of genic heterozygosity. The widespread use of electrophoresis in recent years and the discovery of extensive molecular polymorphism has prompted interest in the adaptive significance of enzyme polymorphism[13,14]. Surprisingly little attention has been paid to the potential role of this enzyme heterozygosity in developmental homeostasis.

The results presented here suggest that genetic heterozygosity and perhaps enzyme heterozygosity *per se* are associated with developmental homeostasis in natural populations. However, the observation also involves a serious paradox. As each monarch butterfly is potentially heterozygous for thousands of such loci, all contributing in a similar fashion, it seems unreasonable to expect to observe this effect for any random set of six loci. Over the entire genome each individual should have approximately the same average heterozygosity, and the specific contribution of any single locus should be obscured. It must, therefore, be assumed that either these particular six loci possess unexpectedly strong contributions, or that they serve as indices of overall genetic heterozygosity. There is no reason to attribute special effects to these enzymes, but homozygosity could be associated across many loci, either through inbreeding (inbred individuals will be concurrently homozygous for many loci), or through linkage disequilibrium of large blocks of loci. No evidence can be found for significant levels of inbreeding in the monarch population[4]. There is already ample evidence from several studies in *Drosophila* that electrophoretic variants can be in strong linkage disequilibrium with linked chromosomal inversions[15-19]. Unfortunately nothing is known about the plausibility of inversion polymorphism in Lepidoptera.

The results of this study are important because they help to generalise the observation that morphological variability and genetic heterozygosity are related in natural populations. While it is not clear what role enzyme polymorphism of the type identified by electrophoresis plays in this association, it is apparent that natural selection, by removing the morphologically extreme, will favor heterozygosity at several of these enzyme-loci[20,21].

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Humans and apes are genetically very similar

MAN'S closest living relatives are the great apes—the African chimpanzee and gorilla, and the Asian orangutan. Humans (*Homo*) are classified in the family Hominidae; chimpanzees (*Pan*), gorillas (*Gorilla*) and orangutans (*Pongo*) are classified in the family Pongidae. The gibbons, or lesser Asian apes (genera *Hylobates* and *Symphalangus*), are classified in the family Hylobatidae. All three families of humans and apes are included in the superfamily Hominoidea. Linnaeus recognised the basic morphological similarities between humans and apes by placing them together in the order primates (Anthropomorpha), although in separate genera. He did not use any category of classification intermediate between 'genus' and 'order'. However, when the 'family' category was introduced, humans and apes were placed in separate families. The prevailing view of modern taxonomists has been expressed by Simpson[1]: "*Homo* is both anatomically and adaptively the most radically distinctive of all hominoids, divergent to a degree considered familial by all primatologists". With the methods of classical mendelian genetics it is impossible to make genetic comparisons between species which cannot be hybridised, as the presence of genes is ascertained by studying segregation in the progenies of crosses between individuals exhibiting different traits. However, advances in molecular biology have made possible comparisons between DNA sequences or, more often, between proteins from different organisms. If the proteins are identical in amino acid sequence, the genes coding for them are assumed to be identical. (This is not always true owing to the redundancy of the genetic code, but DNA nucleotide differences which change the encoded protein have greater evolutionary consequences than those which do not.) Many proteins from different organisms can be readily compared by means of gel electrophoresis. Although not all amino acid differences are detected by this method, it provides reasonably satisfactory estimates of the relative degree of genetic differentiation between closely related species. King and Wilson[2] studied electrophoretically 44 gene loci coding for proteins in humans and chimpanzees (*Pan troglodytes*), and found them to be about as similar as very closely related species of other organisms are to each other. We now report results confirming that finding and extend it to comparisons between humans and the other apes.

Using polyacrylamide and starch gel electrophoresis, we have studied 23 gene loci coding for blood proteins in humans (*Homo sapiens*), the two species of chimpanzee (*P. troglodytes* and the pygmy chimpanzee, *P. paniscus*), the gorilla (*Gorilla gorilla*), the Sumatran and the Bornean orangutans (*Pongo pygmaeus abelii* and *P. p. pygmaeus*), and three species of gibbons (*Hylobates lar*, *H. concolor* and *Symphalangus syndactylus*. Table 1 gives the genetic identity and the genetic distance for the comparisons between humans and each of these organisms. The genetic identity, I, may vary between 0 (complete genetic differentiation at every locus) and 1 (complete identity at every locus). The genetic distance, calculated as $D = -\log_e I$, may vary between 0 and ∞, and estimates the number of electrophoretically detectable allelic substitutions that have occurred in the evolution of two species since their last common ancestor[3]. Table 1 also gives the number of individuals studied for each ape species. The 10 gorillas are unrelated wild-born animals; all other animals are either wild-born or unrelated to each other.

Humans are genetically about equally differentiated from all the great apes; on average, about 35.4 electrophoretically

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Table 1 Genetic identity, *I*, and genetic distance, *D*, between humans and various apes

	No. of loci*	No. of individuals	*I*	*D*
Pan troglodytes	22	23	0.680	0.386
Pan paniscus	23	4	0.732	0.312
Gorilla gorilla	22	10	0.689	0.373
Pongo pygmaeus abelii	23	8	0.710	0.347
Pongo pygmaeus pygmaeus	21	3	0.705	0.350
Hylobates lar	21	4	0.489	0.716
Hylobates concolor	21	2	0.429	0.847
Symphalangus syndactylus	21	1	0.333	1.099
Averages†				
Great apes (1–5)	23	48	0.702 ± 0.009	0.354 ± 0.013
Lesser apes (6–8)	21	7	0.417 ± 0.045	0.887 ± 0.112
All apes (1–8)	21	55	0.595 ± 0.055	0.554 ± 0.105

* The proteins studied were adenosine deaminase, adenylate kinase, catalase, fumarase, glucose-6-phosphate dehydrogenase, glutamate oxaloacetate transaminase, haemoglobin $\alpha\beta$, isocitrate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, NADH-diaphorase, phosphoglucoisomerase, phosphoglucomutase, 6-phosphogluconate dehydrogenase (red-cell proteins), albumin, alkaline phosphatase, ceruloplasmin, esterase, haptoglobin, leucine aminopeptidase (plasma proteins). The following are omitted: adenosine deaminase in the gorilla and the three lesser apes, catalase in the three lesser apes and fumarase in *P. troglodytes*.

† Arithmetic means with their standard errors.

detectable allelic substitutions have occurred for every 100 gene loci in the separate evolutions of man and each of the apes. As expected, the genetic differentiation is larger between humans and the lesser apes, but even here the degree of genetic differentiation is relatively small; on average, there have been about 88.7 allelic substitutions per 100 loci.

The significance of these results becomes apparent when they are compared with electrophoretic data obtained in other animal groups. The average genetic distances (calculated from published data, mostly in ref. 4) between pairs of closely related species are as follows: *Drosophila* flies, $D = 0.827$ (8 species); fish, $D = 0.622$ (4 species); salamanders, $D = 0.296$ (3 species); lizards, $D = 0.828$ (2 species); rodents, $D = 0.359$ (7 species). Although estimates of D are subject to large potential errors (typically, the standard error for a comparison based on 20–30 gene loci may be nearly half as large as the estimate), we may conclude that humans and apes (particularly the great apes) are genetically about as different from each other as closely related species of the same genus in other groups of organisms.

The genetic distance between man and *P. troglodytes* obtained by King and Wilson was $D = 0.62$, about 60% greater than the value obtained by us ($D = 0.386$). Although the two estimates are not significantly different from each other, it is worthwhile to examine the basis for such difference. King and Wilson studied 44 gene loci, whereas we have surveyed only 22 loci in these two species, 18 of which were included in their study. The difference in the two estimates of genetic distance could be accounted for, at least in part, if on average the loci surveyed by them but not by us were more different than the rest, or if the loci surveyed by us but not by them were less different than the rest. This is not, however, the case: when only the loci common to both studies are included in the estimation, the values of D remain approximately as different as for the complete samples. Therefore, the main reason for the higher estimate obtained by King and Wilson is that different electrophoretic methods were used in the two studies, and their methods must have allowed them to detect a greater number of differences.

Three observations should be made. First, the electrophoretic methods used with non-primate species are more similar to our methods than to those of King and Wilson (in fact, many of the studies have been carried out in our own laboratory); thus, our D value is more appropriately used when comparing the genetic differentiation between man and apes with that between other organisms. Second, the use of the D value obtained by King and Wilson does not alter the qualitative conclusion reached that man is genetically no more different from the great apes than are closely related (congeneric) species in other animal groups from each other. (And less different than genera of the same family are in such organisms; for these, we have obtained the following D values: fish, 1.772; salamanders, 0.828; rodents, 1.833.) Third, electrophoretic methods do not detect all amino acid differences between proteins and thus underestimate genetic differentiation; however, this bias should be similar in primates and in other animals and therefore would not affect the validity of the conclusion reached.

The results presented here lead to a paradox. We perceive ourselves as being considerably different in morphology and behaviour from the apes, and this can hardly be exclusively attributed to the fact that humans more readily notice differences involving themselves (indeed, see the statement by Simpson[1] quoted above). However, we have obtained estimates of genetic differentiation between humans and the great apes no greater than, say, those observed between morphologically indistinguishable (sibling) species of *Drosophila* flies. One possible resolution of this paradox is to assume that the estimates are biased; there are thousands of structural gene loci and the few surveyed might not represent a random sample of the whole genome. This is indeed possible but there is no reason to suspect that the sample is biased. Another, more interesting alternative, has been suggested by King and Wilson[2] as well as by others[5–7]. It may be that morphological and behavioural evolution involves primarily changes in gene regulation rather than changes in structural gene loci (that is, those coding for proteins). If this is so, substantial differences in genetic regulation would exist between humans and apes. We favour this alternative. Although this hypothesis is not supported by any direct evidence, it is favoured by some indirect evidence[1] as well as by the demonstration in *Drosophila* that adaptation may occur through changes in regulatory rather than in structural genes[8].

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Totipotent cells of parthenogenetic origin in a chimaeric mouse

IN strain LT/Sv mice, eggs often begin to develop parthenogenetically in the ovary after the first meiotic division[1-3]. They undergo apparently normal cleavage and blastocyst formation, but at the egg cylinder stage they become disorganised and form teratomas. They persist as tumours composed of many kinds of tissue, indicating that cells of parthenogenetic origin are viable. Spontaneous parthenogenesis is also common in ovulated strain LT eggs. They cleave, form blastocysts and implant in the uterus, but die shortly afterwards, in the same way that embryos derived from experimentally induced parthenogenesis die early[4-7]. However, parthenogenetic cells have been 'rescued' by removing embryos from the oviducts of virgin females and grafting them to extrauterine sites such as testis[1] and kidney[8]. Those embryos become disorganised and can survive as teratomas composed of many kinds of viable tissues. Why, then, do cells of parthenogenetic origin fail to survive *in utero*? To investigate this problem we are using chimaeras derived from aggregates of eight-cell parthenogenetic and normal embryos[2], transferred to the uteri of pseudopregnant females. We report here that early embryonic parthenogenetic cells are totipotent. They can give rise to fully functional ova which, when fertilised by sperm, develop into normal individuals.

Parthenogenetic four- and eight-cell embryos of the pigmented strains LT/Sv and LTXBJ and their F_1 hybrids, and embryos derived from normally fertilised eggs of albino strain 129/Sv and (129 × A/He)F_1, were flushed from the oviducts of superovulated females. After removal of the zona pellucida with Pronase, one normal and two parthenogenetic embryos were aggregated in Whitten's[9] medium in an atmosphere of 5% CO_2, 5% O_2 and 90% N_2 at 37 °C. The aggregated 'triplets' were transferred in groups of about 10 to the uteri of pseudopregnant (SJL × C57BL/10)F_1 females; 456 aggregates were transferred to the uteri of 47 females. Twenty-four offspring were produced and six were identified as chimaeras by their partially pigmented coats and irises (Table 1). Four male and two female parthenote ↔ normal chimaeras were produced. All were mated with strain 129 albinos. The four males sired 285 offspring, all albinos. Chimaera no. 19 (129 × A/He)F_1 ↔ (LT × LT × BJ)F_1 produced 33 albino offspring. Chimaera no. 53 (129 ↔ LT) produced five litters of 29 albino offspring. Her sixth and last litter contained two albino and two (one male and one female) pigmented offspring (Fig. 1). The pigmented offspring could only have been derived from ova of parthenogenetic origin (LT) fertilised by normal albino (129) sperm.

We were able to identify the parthenogenetic origin (LT) of the ova of chimaera no. 53 by a genetic marker other than *C*, which determined the pigmented coats of her offspring. Strain LT is Hbb^s/Hbb^s, which produces an electrophoretically single haemoglobin, and strain 129 is Hbb^d/Hbb^d, which produces a diffuse electrophoretic pattern[10]. Cellulose acetate electrophoresis of mouse haemoglobins modified with the disulphide reagent cystamine permits rapid, unequivocal discrimination of all combinations of the co-dominant mouse haemoglobin 'single' (Hbb^s) and 'diffuse (Hbb^d) alleles[11]. Examination of the blood of the two pigmented offspring revealed that they were heterozygous Hbb^s/Hbb^d. The Hbb^s allele could only have been derived from ova of parthenogenetic LT origin.

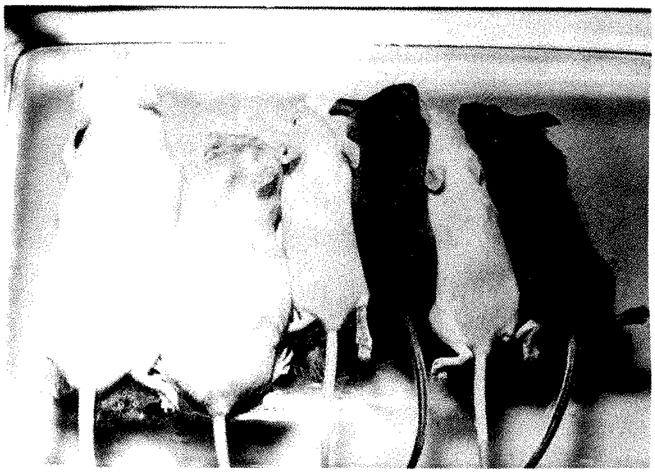

Fig. 1 An albino male (left) was mated to a chimaeric female (second from left) derived by aggregating normal and parthenogenetic 8-cell embryos. This mating produced a litter with two albino (derived from normal eggs) and two pigmented (derived from eggs of parthenogenetic origin) young.

Two of the chimaeric neonates were about half the size of their normal littermates. This suggested the possibility that in aggregates with parthenogenetic cells there is extensive cell death. To increase the possibility of having more parthenogenetic cells per embryo, parthenogenetic embryos were aggregated with other parthenogenetic embryos and transferred to the uteri of pseudopregnant females; 55 aggregates were transferred and five were recovered after implantation.

One female received nine aggregates of four parthenogenetic four-to-eight-cell embryos. Two abnormal embryos implanted; one had only a small amount of ectoderm undergoing necrosis and abnormally arranged parietal endodermal cells. The other was at the 6-d stage. It was larger than normal but the ectoderm was irregularly arranged.

Another female received 10 aggregates of three parthenogenetic embryos. There were three implantations; two were at the 5-d stage, with proamniotic cavities lined by dying ectodermal cells; the third was more advanced, but very abnormal. It had a primitive streak, chaotically arranged mesodermal cells,

Table 1 Production of chimaeras by aggregating normal and parthenogenetic embryos

Normal embryos (*c/c*)	Parthenogenetic embryos (*C/C*)	No. of aggregates transferred	No. of host females	No. born per litter	No. of chimaeras*
129/Sv	LT/Sv	160	16	2 born dead	?
				3	1 (53)
(129 × A/He)F_1	LT/Sv	113	11	4	0
129/Sv	(LT/Sv × LTXBJ)F_1	96	10	2	0
				4	3 (341, 42, 43)
129/Sv	LTXBJ	7	1	0	0
(129 × A/He)F_1	(LT/Sv × LTXBJ)F_1	80	9	2	1 (137)
				4	1 (19)
				3	0
		456	47	24	6

* Numbers in parentheses refer to designated chimaera no.

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and an abnormal amnion, allantois and chorion. Although spontaneous parthenotes rarely develop to such an advanced stage, it is not certain that the advanced development was due to the increased number of cells produced by the aggregation of three embryos. These results suggest that increasing the number of cells in a parthenogenetic embryo will prolong its survival *in utero*.

Even though pure parthenogenetic embryos will not survive *in utero*, these results demonstrate that their cells are capable of participating in normal development when associated with normal cells. Furthermore, the production of offspring with the *C* and *Hbb*^{s} alleles from an aggregation chimaera between a parthenogenetic *C/C Hbb*^{s}*/Hbb*^{s} and a normal *c/c Hbb*^{d}*/Hbb*^{d} embryo demonstrates that fully functional totipotent ova of parthenogenetic origin were produced.

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Note added in proof: Illmensee[12] transplanted single ovarian teratocarcinoma cells into genetically different blastocysts and obtained chimaeric mice with internal organ mosaicism; in one case the tumour cells populated the germ line and developed into functional eggs, giving rise to healthy offspring.

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Genetic regulation of glucose phosphate isomerase in mouse oocytes

THE enzyme glucose phosphate isomerase (GPI; D-glucose-6-phosphate-ketol isomerase EC 5.3.1.9.) catalyses the interconversion of fructose-6-phosphate and glucose-6-phosphate. In mice, the product of the GPI structural gene is a monomer and the enzymatically active dimer results from monomer aggregation[1,2]. In inbred strains, GPI is found in either of two electrophoretically distinct forms: GPI-1AA or GPI-1BB (ref. 2). Theoretically, the random association of equal numbers of A and B monomers will result in dimers in the proportion of 25%AA:50%AB:25%BB, and indeed an activity distribution of these proportions is observed when samples of purified AA and BB forms, equal in enzymatic activity are dissociated and reassociated *in vitro*[3]. It follows, then, that (1) monomer aggregation is a random process which does not distinguish the monomer types; (2) the electrophoretic forms of GPI are similar enzymatically; (3) the measured activity is linearly proportional to the amount of enzyme. Therefore, the 25%AA:50%AB:25%BB activity distribution of GPI found in the mature tissues of heterozygous mice (*Gpi-1*$^{a/b}$) (ref. 4) indicates that the *a* and *b* alleles are equally expressed. However, we show here that this is not the rule for unfertilised ova, for we have observed differences in the expression of GPI in unfertilised ova from the inbred mouse strains DBA/2J, LP/J and C57BL/6J. Mature somatic tissues do not reveal these interstrain differences. We postulate that the amount of GPI in ova is controlled by a *cis*-active regulator gene linked to the GPI structural locus. This is apparently the first characterisation of a genetic variation affecting the amount of an enzyme in the

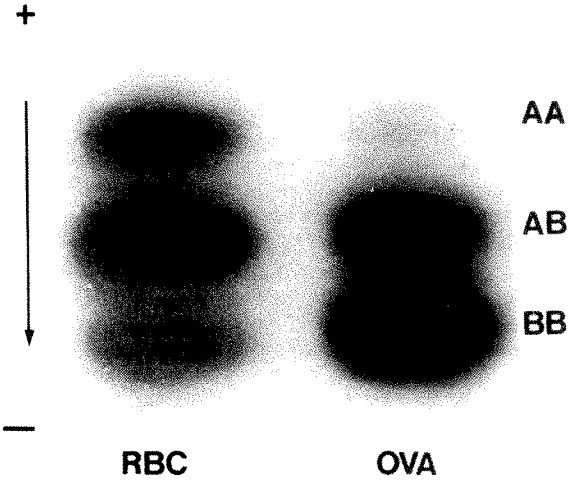
Table 1 GPI activity in unfertilised ova

Female type from which ova obtained	GPI genotype	GPI activity (nmol per h per ovum ± s.d.)
DBA/2J	*Gpi-1*$^{a/a}$	0.36 ± 0.11
C57BL/6J	*Gpi-1*$^{b/b}$	1.59 ± 0.20
LP/J	*Gpi-1*$^{a/a}$	2.08 ± 0.22
B6D2F$_1$*	*Gpi-1*$^{a/b}$	1.07 ± 0.08
D2B6F$_1$†	*Gpi-1*$^{a/b}$	0.76 ± 0.08

Ova were obtained from mature females in which ovulation had either occurred spontaneously or had been primed (3 IU pregnant mare's serum followed 45 h later with 3 IU human chorionic gonadotropin). 8–12 h post-ovulation, the females were killed and their ova were released into Brinsters medium (Gibco) by flushing the fallopian tubes. Adhering cumulus cells were removed by incubating the ova in hyaluronidase (Sigma type IV, 75 units ml^{-1}). The ova with intact zona pellucidae were washed three times in Brinsters medium. For spectrophotometry, the zona pellucidae were removed by incubation in Pronase (0.5%) followed by three washes in Brinsters medium. The ova were recovered either singly or in groups in approximately 1 µl of medium in glass pipettes. Before analysis, these samples were stored at −80 °C for up to 4 weeks. The activity of GPI was determined spectrophotometrically[13] using pooled samples of up to eight ova. The assay mixture consisted of: 0.3 ml Tris HCl buffer, *p*H 8.0 (0.1 M Tris); glucose-6-phosphate dehydrogenase, type XII, from Torula yeast, 1 unit ml^{-1}; D-fructose-6-phosphate (Sigma, grade I) 4.65 mM; NADP, sodium salt, 2.25×10^{-1} mM. Three replicate assays per vum type were carried out at room temperature in a Guilford, model 250, spectrophotometer. The rate of the reaction was constant and linear during the assay period (25 min). All values, as assessed by the *t* test, are significantly different (<0.05) from their nearest neighbour. B6D2F$_1$ and D2B6F$_1$ ova were expected to have intermediate and similar activities. Ova from these reciprocal F$_1$ hybrids had GPI-1 activities between the two parental classes but they were not identical. The relatively small difference in GPI-1 activity between the two types of F$_1$ hybrids may result from a maternal effect, but this requires further investigation.

* B6D2F$_1$ female; progeny of C57BL/6J female and DBA/2J male.
†D2B6F$_1$ female; progeny of DBA/2J female and C57BL/6J male.

Fig. 1 GPI phenotype in red blood cells (RBC) and ova. Starch gel electrophoretogram[4] demonstrating GPI expression within a heterozygous (*Gpi-1*$^{a/b}$) B6D2F$_1$ mouse. GPI in RBC demonstrates the expected proportions (25%AA:50%AB:25%BB) whereas ova demonstrate a pattern skewed in favour of GPI-1BB (3.4%AA:29.9%AB:66.8%BB). To assist in photography of the AA band, two identical ova were combined for this sample.



Parental strains	C57BL/6J *Org*$^{high/high}$ *Gpi-1*$^{b/b}$	×	DBA/2J *Org*$^{low/low}$ *Gpi-1*$^{a/a}$
Heterozygous F_1 progeny		B6D2F_1 *Org*$^{high/low}$ *Gpi-1*$^{b/a}$ Ova phenotype: [A]:[B] = 21:79	
Backcross	C57BL/6J *Org*$^{high/high}$ *Gpi-1*$^{b/b}$	×	B6D2F_1 *Org*$^{high/low}$ *Gpi-1*$^{b/a}$
Heterozygous BC_1 progeny		If recombination occurs *Org*$^{high/high}$ *Gpi-1*$^{a/b}$	If no recombination occurs *Org*$^{high/low}$ *Gpi-1*$^{b/a}$
	Ova phenotype:	[A]:[B] = 50:50	[A]:[B] = 21:79
No. of progeny	0	65	

Fig. 2 Gene mapping study. The optimal circumstance for comparing the relative expression of the two *Gpi-1* alleles is to investigate their products in the same ova. The electrophoretic system used here permits that analysis (see Table 2). Therefore, ova from only *Gpi-1*$^{a/b}$ backcross progeny were examined. The electrophoretograms (of the BC_1 ova) were scored visually. A recombinant would demonstrate the typical RBC pattern whereas a nonrecombinant would demonstrate the ova pattern presented in Fig. 1. *Org* represents the putative site responsible for the genetic variation of GPI activity in ova. From Table 1, we can designate *Org* as high for C57BL/6J and as low for DBA/2J

mouse ovum and it provides a unique opportunity for further exploration of gene regulation during mammalian development.

Ova were obtained from the mouse strains DBA/2J, C57BL/6J and LP/J, and their GPI activities were determined spectrophotometrically. Table 1 shows that these ova express significantly different GPI activities, which do not seem to be correlated with the electrophoretic phenotype of GPI, as both LP/J and DBA/2J are homozygous for the same electrophoretic allele (*Gpi-1*^{a}). Nevertheless, relevant differences in the structural gene cannot be excluded on this basis alone, because the GPI structural genes in these strains could differ in ways which do not affect the electrophoretic mobility of the protein product.

As a first step in uncovering the mechanism responsible for these differences in GPI activity, ova from reciprocal F_1 hybrids between C57BL/6J (*Gpi-1*$^{b/b}$) and DBA/2J (*Gpi*-1$^{a/a}$) were examined. Although significantly different, their enzyme activities were found to be intermediate between those of ova from the parental strains (Table 1). In these F_1 ova, the expression of the two alleles may be under coordinate or individual control (see ref. 5). Coordinate control would lead to the equal expression of *a* and *b* alleles; if the two alleles were controlled individually, however, we would predict from the spectrophotometric data of the parental strains that the activity distribution would be skewed in favour of GPI-1B (Table 2). Figure 1 shows that the activity distribution is skewed. The ratio of A to B monomers is not unity but matches the ratio of the oocyte activity of the respective parental strains, indicating that in the maturing oocyte the *a* and *b* alleles are under individual control.

The electrophoretic distribution of GPI activity in all of 44 individual F_1 ova (16 ova from six B6D2F_1 females and 28 ova from seven D2B6F_1 females) was identical, indicating that the GPI phenotype of an unfertilised ovum is independent of both its prospective haploid genotype[6] and its maternal origin. Therefore, our results are consistent with a *cis*-regulation model which predicts that the major genetic variation, responsible for strain differences of ova GPI activity, lies within either the GPI structural gene or a closely linked controlling gene. We propose that this genetic variation defines a new regulator gene which we name *Org* for oocyte regulation of GPI.

To determine more precisely the site of the proposed *Org* locus, a gene-mapping experiment was carried out (Fig. 2); no recombination event between *Org* and *Gpi-1* was observed in 65 backcross progeny. Therefore, if we assume that recombination between *Org* and *Gpi-1* is possible, the upper limit (with 95% confidence) for the map distance separating the two is 4.5 map units[7].

Of the many possible mechanisms for the action of *Org*, differential degradation of dimers seems unlikely for two reasons. First, we would predict that the relative activities of GPI-1AA and GPI-1BB in hybrid ova would be similar to those observed between the ova of the parental strains (that is, 19:81) if a difference in the stability of the dimers, in the ova cytoplasm, were involved. However, the electrophoretic activity distribution of GPI closely approximates that expected if the monomers were being produced in the ratio predicted from parental activities, and not that expected from degradation of dimers. The second reason is provided by the *Gpi-1*^{c} allele. Mice homozygous for this allele produce a GPI-1CC dimer which is electrophoretically distinct from GPI-1AA and GPI-1BB forms, and the GPI-1CC homodimer is unstable. The mouse red blood cell is expected to show little or no protein synthesis and therefore is a useful preparation for examining relative protein stability[8]. In the red blood cells of mice heterozygous for the *Gpi*-1^{c} allele, although the three expected bands are present, there is relatively little activity in the GPI-1CC band[8]. However, red blood cells from reciprocal hybrids between C57BL/6J and DBA/2J strains do demonstrate the typical 25%AA;50%AB:25%BB pattern (Fig. 1) which suggests that the dimers in these mice have equal stability.

Our findings describe a genetic variation affecting the expression of an enzyme during oogenesis in the mouse. Although the enzyme profile of unfertilised ova and preimplantation embryos from 'random bred Swiss' mice has been intensively studied[9,10], ova from inbred strains have not been similarly examined. In the only other study similarly comparing ova from different inbred

Table 2 Expression of *Gpi-1* alleles in F_1 ova

Ova obtained from female of genotype	Predicted ratio of A to B monomers, if *Gpi-1*^{a} and *Gpi-1*^{b} are Coordinately* controlled	Individually† controlled	The ratio of A to B monomers determined by electrophoresis‡
B6D2F_1	50:50	19:81	21:79
D2B6F_1	50:50	19:81	18:82

* If coordinately controlled, both *Gpi-1* alleles would be equally expressed and therefore both A and B monomers will be present in equal amounts.

† From Table 1 the ratio of GPI activities of DBA/2J:C57BL/6J is 19:81. If individually controlled, the *Gpi-1* alleles would be unequally expressed and therefore A and B monomers will be present in unequal amounts, in the ratio of 19:81.

‡ Using a sensitive filter overlay technique for recovering an indirect reaction product of the GPI enzyme, it is possible to quantitate patterns on starch gel electrophoretograms[4]. The observed proportions (±s.d.) were, for B6D2F_1 ova: 5.3 ± 1.5 AA; 31.8 ± 3.0 AB; 62.9 ± 3.4 BB and for D2B6F_1 ova· 3.4 ± 1.7 AA; 29.8 ± 3.0 AB; 66.8 ± 4 5 BB. As the monomer compositions of the three GPI electrophoretic forms are known, it is possible to calculate the ratio of A monomers to B monomers for any given electrophoretogram. Let [A] and [B] represent the quantity of the respective GPI monomer types and [AA], [AB] and [BB] represent the amount of the dimer types, then the ratio of A:B monomers is ([AB] + 2[AA]):([AB] + 2[BB]).

strains, small but significant interstrain differences in lactate dehydrogenase activity were observed[11]. Therefore, it seems that biochemical data on the ova of random bred Swiss mice may not be generally representative.

We conclude that the absolute level of ova GPI activity, although strictly regulated within each strain, can vary over a considerable range and yet permit successful reproduction. This situation now provides a unique opportunity for further exploration into developmental gene regulation during oögenesis and also during preimplantation development[12,13].

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A better cell line for making hybridomas secreting specific antibodies

FUSION of myeloma cells which grow in tissue culture with spleen cells from an immunised mouse provides a general method for obtaining cell lines (hybridomas) which make antibody of the desired specificity[1–3]. Hybrids derived from these myelomas make the immunoglobulin (Ig) heavy and light chains of the myeloma parent as well as the antigen-specific heavy and light chains of the spleen cell parent. In conditions in which the two heavy and two light chains associate randomly, a hybridoma would make 10 distinct Ig molecules, and the specific antibody would comprise only 1/16 of the total Ig[4,5]. To obtain hybridomas making only the specific antibodies requires a tumour cell fusion partner that itself makes no Ig but which can nevertheless be fused with spleen cells to obtain hybrids secreting only the specific antibody. We report here the identification of such a cell line, Sp2/0-Ag14.

Sp2/0-Ag14 was isolated as a re-clone of Sp2/HL-Ag, itself derived in several steps from Sp2/HLGK, a hybrid between a BALB/c spleen cell contributing a $\gamma 2b$ (H) and κ (L) chain with anti-sheep red blood cell activity and the myeloma cell line X63-Ag8 (γ (G) and κ (K))[2]. Sp2/0-Ag14 is resistant to 20 μg ml^{-1} 8-azaguanine, dies in HAT supplemented medium and synthesises no Ig chains. It has about 73 chromosomes which is only eight more than the chromosome number of X63-Ag8, a cell line commonly used to generate hybridomas.

Sp2/0-Ag14 was fused with spleen cells of mice immunised with trinitrophenyl derivatised keyhole limpet haemocyanin (TNP-KLH) to generate hybridomas making TNP-specific immunoglobulin (Fig. 1). Fusions were done with either polyethylene glycol (PEG) 1500 or Sendai virus, and transferred to medium either containing or lacking mouse peritoneal exudate cells. Growth was observed in only eight culture wells, all from the set that were fused with PEG and grown with peritoneal exudate cells.

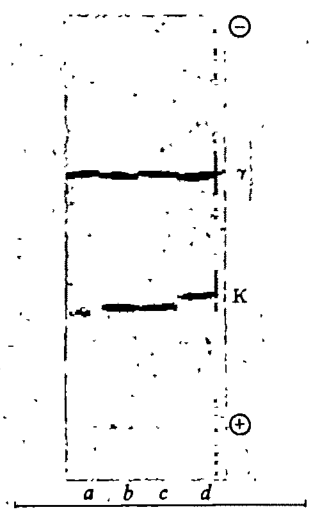

Fig. 1 Immunoglobulin from TNP-specific hybridomas analysed by SDS-polyacrylamide gel electrophoresis. To obtain hybridomas making Ig specific for the hapten trinitrophenyl, BALB/c mice were immunised i.p. with trinitrophenyl-derivitised keyhole limpet haemocyanin (TNP-KLH) (from H. Kiefer), first with 100 μg TNP-KLH contained in 0.2 ml emulsion of 50% phosphate-buffered saline (PBS), 50% complete Freund's adjuvant (Difco). 35 days later a second injection of 100 μg TNP-KLH in 0.2 ml PBS was made i.p., and 5 days later the spleens of these mice were prepared for fusion. Fusion with PEG 1500 was as described by Galfre *et al.*[9], except that the medium used was R medium, RPMI 1640 medium (Gibco) supplemented with 30mM HEPES (Flow). Here 3×10^7 Sp2/0-Ag14 and 5×10^7 spleen cells were centrifuged together at 200 g for 5 min and resuspended slowly in 0.6 ml 50% PEG in Dulbecco's modified Eagle's medium (Flow). After 1 min at 37 °C, 20 ml of R medium was added slowly. The cells were then centrifuged and resuspended in 20 ml of R medium supplemented with 10% fetal calf serum (Gibco) (RF medium) and 0.2 ml of this suspension was then distributed to each of 200 wells containing 0.8 ml RF medium. One hundred of these wells also contained 2×10^5 mouse peritoneal exudate cells. After 24 h incubation, 1 ml RF supplemented with hypoxanthine, aminopterin and thymidine (HAT) was added to each well. Every 2–3 days, 1 ml of the medium was replaced with fresh RF+HAT. After 2 weeks, growth was observed in eight wells of the peritoneal exudate supplemented wells and the cultures were tested for immunoglobulin production by testing the culture medium for lysis of protein A coated sheep red blood cells (SRC) for nonspecific Ig[10] and of TNP coated SRC[2] (for TNP specific activity). After 1–2 months the cells from TNP-specific wells were cloned by plating in soft agar[11] or in methyl cellulose[12]. The soft agar clones were overlaid with TNP coated SRC, and clones lysing the TNP coated SRC were transferred to liquid medium. The cells were grown in the presence of ^{14}C leucine to label secreted Ig which was analysed by SDS polyacrylamide gel electrophoresis as described[11]. The hybridoma cell lines analysed were hy5.19 (*a*), hy2.15 (*b*), hy1.2 (*c*) and hy3.3 (*d*).

Supernatant of four of the eight growth-positive wells lysed protein A but not TNP-coated sheep red blood cells (SRC) suggesting that the Ig made by these cells did not have TNP specificity. The Ig secreted by these cells was analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and found to be IgM (data not shown). The remaining four growth positive wells lysed both TNP and protein A SRC and cells from these wells were cloned and analysed further, as described below. SDS-PAGE analysis suggested that these cell lines secreted IgG (Fig. 1). Ouchterlony analysis of culture supernatants using class-specific antisera (Litton Bionetics) indicated that hy1.2 makes an IgG2a, and that hy2.15, hy3.3 and hy5.19 make IgG1 and that all four TNP-specific hybridomas make a κ light chain. The SDS-PAGE analysis shown in Fig. 1 indicates that the clones can be distinguished by the mobility of the light or heavy chain, implying that these cell lines are making different antibodies.

We have examined the TNP-specific hybridomas for re-expression of the SRC-specific IgG2b of the progenitor cell line Sp2/HL-Ag14 by testing for SRC-specific plaques. No plaques

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were found and we conclude that revertants constitute fewer than one in 5×10^4 of the TNP-specific hybridoma cells.

The karyotypes of the TNP-specific cell lines provide evidence that these cells are hybrids of Sp2/0-Ag14 and mouse cells. Normal mouse cells and Sp2/0-Ag14 contain 40 and 73 chromosomes, respectively. The TNP-specific cell lines hy1.2, hy2.15, hy3.3 and hy5.19 contain approximately 100, 105, 106 and 99 chromosomes, respectively, and we suppose that fusion yielded hybrids that segregated better growing cells that had lost 7–14 chromosomes.

Evidence has been presented that X63-Ag8 cells preferentially fuse with the dividing cells of the spleen[2,6,7], which for the B-cell compartment consist mainly of Ig-secreting, plaque-forming cells. Sp2/0-Ag14 seems to display the same preference. In the population of spleen cells used for this fusion, approximately 0.4% of the cells made indirect (IgG) TNP-specific plaques and 0.004% made direct (IgM) TNP plaques. Assuming that 1% of the spleen cells are plaque forming cells[8] one would anticipate that about 40% of the hybridomas would make IgG with TNP specificity, as was observed. On the other hand, about 90% of the nonspecific plaque-forming cells of the spleen make IgM rather than IgG[8], so that one would anticipate that among the nonspecific hybridomas, 90% would also make IgM, a figure that is consistent with our observation that all four nonspecific hybridomas examined made IgM.

To obtain high-titre preparations of the TNP-specific Ig, 10^6 TNP-specific hybridoma cells were injected intraperitoneally into BALB/c mice. All caused tumours and yielded ascites fluid with titres generally about 100 times higher than for the culture supernatants.

The efficiency of fusion with Sp2/0-Ag14 has been variable. In our early experiments, such as the fusion described above, we obtained fewer hybrids than we ordinarily obtain with X63-Ag8. However, in more recent experiments, fusions with Sp2/0-Ag14 have often been more efficient, in particular in fusions with lipopolysaccharide stimulated spleen cells or with large spleen cells (F. Melchers, Clark and G.K., unpublished observations). We consider that Sp2/0-Ag14 can yield as many hybrids as X63-Ag8.

Alternative methods exist for obtaining hybridomas that do not express the myeloma Ig. It has been possible to isolate re-clones that have lost the expression of the nonspecific heavy or light chains[2]. This problem has been reduced by fusing with NSI-Ag 4/1, a derivative of X63 that makes the κ but not the $\gamma 1$ chain of the myeloma[2]. We anticipate that Sp2/0-Ag14 will prove yet a better cell line for generating hybridomas making truly monoclonal antibodies.

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Activation of latent Epstein–Barr virus by antibody to human IgM

EPSTEIN–BARR VIRUS (EBV) is the causative agent of most infectious mononucleosis[1] and is also associated with two human tumours, Burkitt's lymphoma (BL)[2] and nasopharyngeal carcinoma[2]. Lymphoid cell lines of B-cell origin have been established from patients with these diseases, and from lymphocytes transformed *in vitro* by EBV[3]. In lymphoid cell lines the lytic viral cycle is usually repressed even though the cells carry multiple copies of the EBV genome. These cells therefore provide a valuable model for the study of latent EBV infection. Although EBV is ubiquitous, its association with human malignant disease is to a large extent both ethnically and geographically restricted[2] and it is therefore of interest to understand the host factors involved in the regulation of the expression of the latent EBV genome. In infectious mononucleosis, EBV infection is associated not only with a specific anti-EBV antibody[2] and T-lymphocyte response[4] but also with an increase in nonspecific IgM production[5]. EBV-infected B lymphocytes produce polyclonal Ig (ref. 6) and the majority of established EBV genome-positive lymphoid cell lines possess surface IgM (ref. 7). We report here that treatment of several human lymphoid cell lines with antisera to human IgM activates the latent EBV genome to give a marked increase in EBV-specific early antigen (EA). Also, in some cell lines this treatment induces an increase in virus capsid antigen (VCA).

Treatment of BL-derived Raji cells with antiserum directed against human immunoglobulins increased the number of cells expressing EBV EA by up to 5,000-fold, to give 11% EA-positive cells determined by either direct or indirect immunofluorescence (Table 1), and confirmed by EA-specific complement fixation[8]. The fluorescence was specific for EA, as no positive cells were observed after staining with either control EBV-negative human serum, or with anti-VCA-positive EA-negative antiserum. EBV-specified antigens can be induced in lymphoid cell lines by halogenated pyrimidines[9,10], inhibitors of protein synthesis[11] or the tumour promotor 12-*O*-tetradecanoyl

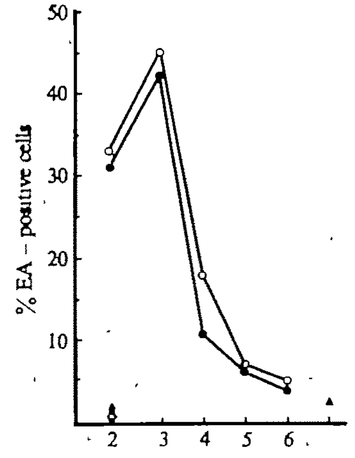

Fig. 1 Induction of EA in cultures of Raji cells treated with various dilutions of antiserum. Cells were cultivated for 72 h with a particular dilution of antiserum in the presence of IUdR ($25 \mu g\ ml^{-1}$). The number of EA-positive cells was then determined on acetone-fixed smears by indirect immunofluorescence. None of the antisera had any effect on cell viability at the dilutions shown in the figure. ○, Sheep anti-total human immunoglobulins; ●, goat anti-human IgM; □, goat anti-human IgA; ■, goat anti-human IgG; △, goat anti-human IgD; ◇, goat anti-human IgE; ▲, IUdR alone ($25 \mu g\ ml^{-1}$)

Table 1 The effect of anti-human immunoglobulins on the expression of EBV-specified antigens

				% EA or (VCA) positive cells			
Cell line	Expt no.	Time (h)	Antiserum dilution	Control	Antiserum treated	IUdR treated	Antiserum + IUdR treated
Raji	1	23	10^{-2} Anti-total	0 002	4.5	0.1	21.0
		47	immunoglobulins	0.006	3.0	0.6	32.0
		71		0.014	7 0	0.7	36.0
	2	20	10^{-2} Anti-IgM	0.002	11.3	—	—
	3	70	10^{-3} Anti-IgM	0.014	2.6	1.8	51.0
	4*	47	Anti-IgM antiserum	—	—	0.26	10.3
			Eluate	—	—	0.26	14.5
			Unbound material	—	—	0.26	0.35
	5†	22	10^{-3} Anti-IgM antiserum	0.014	4 8	0.016	10.0
Daudi	1	44‡	10^{-2} Anti-IgM	0.12	9.0	10.0	21.0
				(0.016)	(0.13)	—	—
	2	49‡	10^{-2} Anti-total	0.01	10 0	4.8	15.0
			immunoglobulins	(0.004)	(0.36)	—	—
BJAB-B95-8	1	23‡	10^{-2} Anti-total immunoglobulins	0 002	0.032	0.004	11.0
P3HR-1	1	47‡	10^{-2} Anti-IgM	6.5	14.0	16.0	22.0
				(5.5)	(7.0)	(14.0)	(17.0)
M61	1	44	10^{-2} Anti-IgM	3.0	2.6	3 5	3.0

Lymphoid cells were cultivated in RPMI 1640 medium with 20% fetal calf serum (Gibco) in static suspension culture for the times indicated with medium alone; medium + IUdR (25 μg ml^{-1}); the dilution of antiserum indicated; or IUdR + antiserum EA or VCA was determined at this time in duplicate on 50,000 cells (fixed with acetone) either by direct immunofluorescence using FITC-conjugated EA(+) VCA(+), or EA(−) VCA(+) human sera, or by indirect immunofluorescence using EBV-specific human antisera and FITC-conjugated goat anti-human IgG globulin (Huntingdon).

* Anti-IgM antiserum was adsorbed on a column of immobilised human IgM as previously described[30]. The serum and eluate contained an equivalent concentration of anti-IgM antibody determined by quantitative immunodiffusion[31].

† In this experiment treatment of Raji cells with TPA (20 ng ml^{-1}, Sigma) gave 0.6% EA(+) cells and TPA with IUdR (25 μg ml^{-1}) gave 5.5% EA(+) cells.

‡ IUdR was removed by washing after 24 h treatment and the cells were then resuspended in either medium alone, or medium containing antiserum.

phorbol-13-acetate (TPA)[12]. In optimal conditions treatment of Raji cells with antiserum consistently gave a greater degree of induction of EBV EA than treatment of these cells with either 5-iodo-2-deoxyuridine (IUdR) or TPA (Table 1). Treatment of Raji cells with antiserum in the presence of IUdR gave a synergistic effect resulting in up to 50% EA-positive cells (Table 1).

The active component of the antiserum to total immunoglobulins was found to be anti-human IgM immunoglobulin. Thus, the EA-inducing activity of antiserum to human IgM paralleled that of antiserum to total human immunoglobulin, whereas purified antisera to human IgG, IgA, IgE and IgD were all inactive (Fig. 1).

The activity of the anti-human IgM antiserum was specific for human IgM, as the EA-inducing activity of the antiserum was removed after passage through a column of immobilised IgM and could be recovered after elution of the column (Table 1). The effect of the anti-IgM antiserum was due to its anti-μ-chain activity, as antisera against the light chains λ or κ were inactive. Normal human, sheep, goat and rabbit sera were all inactive as were antisera against human lipoprotein, β_2 macroglobulin and albumin. Raji cells have been reported to exhibit on their surface, in addition to IgM (ref. 13), a receptor for the Fc fragment of immunoglobulin[14], a receptor for the C3 component of complement[15], HLA histocompatibility antigens[16], β_2 microglobulin[16] and 'Ia'-like antigens[17]. However, no increase in EA-positive cells was observed when Raji cells were treated with complement (either alone or in the presence of anti-C3), anti-β_2 microglobulin antisera, specific anti-HLA antisera or specific anti-Ia antisera. Similarly, treatment of Raji cells with anti-human immunoglobulins in conditions which gave good induction of EA had no effect on the subsequent binding of IgG to the Fc receptor, or aggregated IgG in the presence of complement to the C3 receptor of Raji cells, as determined by the use of anti-human IgG labelled with ^{125}I-*Staphylococcus* protein A (ref. 18) (data not shown).

An EBV-specified DNA polymerase is thought to be one of the virus-coded proteins which constitute EA antigen[19].

Table 2 Incorporation of ^{3}H-thymidine and ^{3}H-IUdR in Raji cells treated with anti-IgM antiserum

Labelled precursor	Sample	c.p.m. per 10^6 cells		Specific activity (c.p.m. per μg DNA)	
^{3}H-thymidine	Control	227,811 ± 17,307	NS	21,048 ± 1,869	NS
	10^{-2} anti-IgM	208,288 ± 58,611		21,212 ± 955	
^{3}H-IUdR	IUdR	27,511 ± 2,515	NS	12,798 ± 1,290	NS
	IUdR + 10^{-2} anti-IgM	25,475 ± 3,310		12,319 ± 1,911	

Raji cells were cultivated in stirred suspension culture for 24 h with either 0.5 μCi ml^{-1} ^{3}H-thymidine (25 Ci mmol^{-1}, CEA) or 0.5 μCi ml^{-1} ^{3}H-thymidine + 10^{-2} dilution of anti-IgM antiserum (Merieux), or 2 μCi ml^{-1} ^{3}H-IUdR (2.7 Ci mmol^{-1}, Amersham) + 25 μg ml^{-1} cold IUdR, or 2 μCi ml^{-1} ^{3}H-IUdR + 25 μg ml^{-1} cold IUdR + 10^{-2} dilution of anti-IgM antiserum. Incorporation of labelled precursors into cellular material was determined by measuring the radioactivity in the trichloroacetic acid-insoluble material retained on a Millipore filter as previously described[32]. The specific radioactivity of purified DNA was determined after extraction of the DNA as previously described[33]. NS, Not significant.

Treatment of Raji cells with anti-IgM antiserum, in conditions which gave a good induction of EA, also caused a marked increase in the activity of the virus-specified DNA polymerase (T. Ooka, G. L., M. G. T., unpublished results). Anti-μ antibody can activate B lympocytes *in vitro*[20,21], leading to antibody production and the stimulation of ^{3}H-thymidine incorporation. However, treatment of Raji cells with anti-μ antibody had no significant effect on either the incorporation of ^{3}H-thymidine or ^{3}H-IUdR into cellular acid-precipitable material or on the specific radioactivity of the purified DNA (Table 2), suggesting that EA induction by anti-μ antibody cannot be explained simply as a nonspecific mitogenic effect.

In agreement with previous reports[22–25], no VCA-positive Raji cells were observed after short-term induction with IUdR or even in cultures treated with anti-IgM antiserum and IUdR, where 50% of the cells were EA positive. Our results suggest that the block in the abortive cycle of Raji cells is after EA and DNA polymerase synthesis and is independent of the level of induction. BL-derived Daudi cells, however, contain surface IgM and are capable of entering a lytic cycle. Treatment of Daudi cells with anti-IgM antibody markedly increased the number of both EA- and VCA-positive cells (Table 1). Treatment of the BL-derived BJAB-B95-8 cell line with anti-IgM antiserum gave up to a 2,000-fold increase in EA-positive cells, although no VCA-positive cells were observed either before or after treatment with antiserum. Some increase in EA (but not VCA) positive cells was obtained in anti-IgM-treated cultures of P3HR-1 cells (Table 1). Anti-IgM antiserum had no effect on the expression of EBV-specified antigens in the lymphoma-derived M61 cell line[25], one of the rare lymphoid cell lines which have surface immunoglobulin predominantly of the IgG class (Table 1). Thus, differences in the amount and/or expression of surface-bound IgM may partly explain variations in the response of different cell lines to the anti-IgM antibody, although the nature of the interaction between viral and cellular genomes is also probably important.

We have shown that anti-IgM antiserum is a more efficient inducer of EBV EA in Raji cells than either of the two most potent chemical inducers, IUdR or TPA, and the use of anti-IgM serum together with IUdR should facilitate the large-scale production of virus-specified antigens and DNA polymerase. However, the main interest of our results is that treatment of lymphoid cells with specific antisera against human IgM leads to activation of the latent EBV genome. The specificity of this interaction (that only anti-IgM antibody activates the EBV genome) suggests that this effect is probably mediated through an interaction of the antiserum with IgM on the cell surface. Activation may result either from a direct stimulation of virus replication by the antiserum, or from combination of the antiserum with surface-bound immunoglobulin which may play a part in the maintenance and perhaps even in the establishment of the latency of EBV infection in human lymphoid cells. It is perhaps relevant that the majority of these cells possess surface immunoglobulin of the IgM class[7]. EBV has been shown to infect B lymphocytes selectively[27] and to stimulate immunoglobulin production in these cells[6]. There is also evidence to suggest that EBV may preferentially transform IgM-carrying lymphocytes[28,29]. It may well be that immunoglobulin production may in turn be related to the regulation of latent EBV infection in these cells. Our results, in addition to demonstrating a very efficient means of inducing EBV-specific antigens, may therefore have some bearing on the mechanism of the latency of EBV infection and its association with human disease.

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Retinoids block phenotypic cell transformation produced by sarcoma growth factor

MURINE SARCOMA VIRUS (MSV)-transformed cells lack available receptors for epidermal growth factor (EGF)[1,2]. We have shown that this altered phenotype is the result of the endogenous production of growth factors by the MSV-transformed cells themselves. The major activity, isolated and purified from transformed cells, has been found in a 12,000-molecular weight peak and competed with EGF in an EGF-receptor-binding assay[3]. This growth factor, called sarcoma growth factor (SGF), stimulates cell division, causes normal cells to grow in soft agar and produces rapid, reversible morphological transformation of cells in monolayer culture[3]. There is no evidence that SGF acts as a complete carcinogen, producing permanent cell transformation; its properties resemble classical chemical promoters of carcinogenesis, like 12-*O*-tetradecanoylphorbol-13-acetate (TPA)[4–6], the highly active component of croton oil. Whereas TPA is an exogenous plant derivative acting on an animal or a cell, SGF is an endogenous,

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Table 1 Effect of SGF and retinyl acetate on the final cell density of rat fibroblast cell cultures

Treatment	Cells per plate ($\times 10^{-6}$) Expt 1	Expt 2
Untreated control	1.2	1.0
+Retinyl acetate (1.9×10^{-8} M)	1.3	1.1
SGF-treated (10 μg ml^{-1})	3.6	2.6
+Retinyl acetate (1.9×10^{-8} M)	1.4	0.9
+DMSO (1:1,000)	3.3	2.5

A subclone (536–7) of a normal rat fibroblast cell clone, NRK 49F (ref. 17), susceptible to the growth-stimulating effect of SGF (ref. 3), was seeded at 2×10^5 cells per plate in 60-mm plastic Petri dishes. The cells were treated at the time of inoculation and refed with medium (Dulbecco's modified medium with 1% fetal calf serum) containing the additions shown every third day. Cell counts were made 10 d after seeding. The SGF came from supernatants of MSV-transformed mouse NIH/3T3 cells as described previously[3]. Retinyl acetate was dissolved in dimethyl sulphoxide (DMSO). The final concentration of DMSO in all experiments was 0.1%. Neither retinyl acetate nor DMSO alone showed any effect on the growth, final cell density or cloning efficiency of the normal rat fibroblasts at the concentrations used.

virally induced growth promoter. In this respect it is interesting that retinoids (vitamin A and synthetic analogues)[7] block the action *in vivo* of exogenous and endogenous promoters, preventing carcinogens from producing new tumours, but do not reverse the growth of many established tumours[7–10]. Retinoids prevent cancer of the lung[7,11], skin[9], bladder[12] and mammary gland[13] in experimental animals, block cell transformation induced by chemicals[14] and radiation[14,15] in culture, and reverse the anchorage-independent growth of transformed mouse fibroblasts[16]. If SGF is part of the natural tumour-promoting system and retinoids are part of the natural defence against that system, then one should be able to demonstrate a direct antagonism in cell culture. This report establishes that this happens.

We have used a subclone (536–7) of a rat fibroblast cell clone (NRK 49F)[17] that showed pronounced morphological transformation and anchorage-independent growth when treated with SGF, forming multiple cell layers and criss-crossing each other in an apparently random fashion. Although the effects are all reversible, these treated cells resemble MSV-transformed cells in their phenotype[3]. The cells which were treated with both SGF and retinoids did not have a disordered growth pattern. Table 1 shows that retinyl acetate, at 6 ng ml^{-1}, almost abolished the growth-stimulatory effect of SGF, as determined by the final cell density reached by the monolayer cultures 10 d after the experiment began. The effect of retinoids on SGF-induced morphological alterations was evident within a few days of treatment.

The retinoid concentrations ($1-2\times10^{-8}$ M) neither reversed the phenotype of virally transformed cells, nor blocked cell transformation produced by transforming viruses, such as the Moloney strain of mouse sarcoma virus (MSV) or simian virus 40 (SV40). Mouse 3T3 cells and rat fibroblasts were tested for susceptibility to transformation by MSV and by SV40. Neither retinyl acetate nor retinoic acid, up to 2×10^{-6} M, could be demonstrated to block either the initiation or the maintenance of virally induced transformation when efficient transforming viruses like MSV or SV40 were used (data not shown). In the same experiment, however, the SGF-induced morphological transformation was inhibited. Retinyl acetate did not inhibit normal cell growth or the cloning efficiency of the rat fibroblast cell clones in Petri dishes (data not shown), but did have a pronounced effect on the final cell ('saturation') density of cells treated with SGF (Table 1). SGF-induced cell growth was blocked and normal growth properties were essentially retained.

Table 2 shows that at concentrations well below those which produce any evidence of toxicity, retinoids prevent SGF-induced colony formation in soft agar. Colonies which did form were smaller and contained fewer cells than those treated with SGF alone. Retinoic acid, retinyl acetate, retinyl methyl ether and retinylidene dimedone were all effective. Cells not treated with SGF and plated in agar remained as single cells with occasional 2–4-cell colonies. The clone used has a spontaneous transformation rate, as determined by agar colony growth, of less than one in 10^6 cells plated. The preparation of SGF used, at 10 μg ml^{-1}, produced 40–50 large colonies per 10^4 treated cells and also many smaller colonies of 4–20 cells. The inhibitory effect of the retinoids was less evident or absent when more active SGF preparations or higher concentrations of SGF were used. Of the compounds tested, retinylidene dimedone was the most efficient inhibitor of SGF-induced phenotypic transformation (Table 2, expt 3). As a control against selective toxicity of retinoids to transformed cells as compared to normal cells, MSV-transformed mouse and rat cells which grow well in agar without SGF were plated in soft agar in the presence of retinyl acetate at 2×10^{-6} M. No reduction in colony-forming ability was seen.

These preliminary experiments establish that, in the system used here, retinoids block the transforming effect of the polypeptide hormone, SGF. Only one concentration of each retinoid, well below the level that produces any cell toxicity, was used, and both the growth promoter and the antagonists were added to the cells at the same time. This system is now available for further studies where the concentrations, duration of treatment and the nature of the interaction between each of the three components (promoter, antagonist and responding cell clone) can be varied in a systematic manner.

The general transformation model we propose has the following features. Viruses and chemical carcinogens act by inducing cells to produce normally repressed or inactive growth-promoting factors. These factors, which may be endogenous or exogenous to given cells, could be important in embryonic development, but if inappropriately expressed later in life could lead to transformation. Tumour viruses either provide transforming genes directly or activate cellular genes; chemical carcinogens do only the latter. These growth-promoting and

Table 2 Effect of SGF and various retinoids on the colony-forming ability of rat fibroblasts placed in soft agar

Treatment	Colonies per plate Expt 1	Expt 2	Expt 3
Untreated controls	0	0	0
+Retinyl acetate (1.9×10^{-8} M)	0	0	0
+Retinoic acid (2.0×10^{-8} M)	NT	0	NT
+Retinylidene dimedone* (1.5×10^{-8} M)	NT	NT	0
+Retinyl methyl ether (2.0×10^{-8} M)	NT	NT	0
SGF-treated (10 μg ml^{-1})	44.5	39.0	49.5
+Retinyl acetate (1.9×10^{-8} M)	2.5	1.5	8.0
+Retinoic acid (2.0×10^{-8} M)	NT	3.2	NT
+Retinylidene dimedone (1.5×10^{-8} M)	NT	NT	0.5
+Retinyl methyl ether (2.0×10^{-8} M)	NT	NT	14.5

On day 0, 1×10^5 rat fibroblast cells, clone 536–7 were treated in monolayer cultures using Dulbecco's modified medium with 1% fetal calf serum as described in the legend to Table 1. On day 2, they were seeded at 1×10^4 cells per plate in 0.3% soft agar containing the additions shown as described previously[3]. All cells not treated with SGF (whether treated with retinoid or not) remained as single cells with occasional (<10%) small colonies of 2–4 cells. Colonies with greater than 20 cells after 2 weeks in agar were scored as positive. NT, not tested

* 5,5-dimethyl-2-retinylidene-1, 3-cyclohexanedione

transforming factors may be produced during early embryogenesis and then 'switched off'. The endogenous viruses, with their capacity to recombine with cellular genes, have the ability to transfer information between cells and presumably within a cell, like bacterial insertion sequences. They may well be vehicles which allow expression of the endogenous growth-promoter structural genes. In this model the promoters, be they endogenous (SGF) or exogenous (TPA) act as proximal effectors of transformation.

In certain interpretations of the two-step theory of carcinogenesis, initiation involves gene damage[4,18]; we suggest that this damage may facilitate the production of normally repressed growth factors and/or their cellular receptors. Exogenous promoters interact with specific membrane components and produce reversible phenotypic effects[6,19,20]. Treatment of cells with either SGF or TPA causes a reduction in the number of available EGF – receptor sites on cells[1,21]. SGF is a peptide hormone structurally similar to EGF; it is an acid- and heat-stable polypeptide with disulphide bonds and with an active component of a molecular weight 12,000 (ref. 3). The diterpenoid TPA (molecular weight 616), on the other hand, is structurally very different[5,22,23], and its effect on promoter-induced phenotypic change and/or competition for available EGF membrane-binding sites is not clear. Initiated cells may already be producing some of their own growth factors but not in sufficient quantity to cause phenotypic transformation. However, additional exogenous promoter would allow a critical concentration for transformation to be reached. A defined system, using cloned cells in culture to study natural tumour promoters and their antagonists, as described here, should permit a more precise analysis of the mechanisms of carcinogenesis.

The virogene–oncogene hypothesis[24] points out the possibly erroneous assumption that virally induced tumours would have to arise through external infection by emphasising that virus-coded or virus-associated genes are already present in several animal species. These genes, rather than the environmentally transmissible agents, are more likely to be involved in the origin of natural cancers. The tumour viruses, although unnatural, in that they have often been selected for producing rapid disease, have provided extremely powerful tools to dissect out and understand the molecular mechanisms involved. Genetically transmitted viral genes and transforming genes are now accepted as part of the normal genetic make-up of many organisms and as being activated by agents such as chemical carcinogens, hormones and radiation[25,26]. In parallel with this is the frequently made assertion that chemical, environmental or even industrial carcinogenesis, are almost interchangeable with one another. The finding that SGF, produced by animal cells themselves, is an extremely potent promoter in cell culture systems suggests that endogenous growth promoters may be significant factors in naturally occurring cancers.

In view of the efficacy with which an endogenous factor such as SGF promotes transformation, natural mechanisms for suppression of transformation must exist. The data presented here, as well as previous *in vivo* studies, indicate that suppressors of transformation, such as retinoids, offer a potential approach to prevent the expression of the transformed phenotype.

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Retinoids as well as tumour promoters enhance deacylation of cellular lipids and prostaglandin production in MDCK cells

IN terms of a multiple-stage model of chemical carcinogenesis, tumour promoters may block differentiation of initiated (somatically mutated) cells and permit sufficient cell proliferation to overcome growth suppression[1,2]. Deacylation of cellular lipids, arachidonic acid metabolism, or both, are biochemical pathways that accompany the effects of tumour promoters and may even mediate cellular differentiation and carcinogenesis[3]. While investigating the effects of several putative anticarcinogenic compounds, we found and report here that retinoids, like the tumour-promoting phorbol diesters, stimulated deacylation of cellular lipids and prostaglandin production in dog kidney

Table 1 Effect of retinoids on PGE_2 and $PGF_{2\alpha}$ production by MDCK cells

Retinoids	Concentration (μg ml^{-1})	No. of dishes	Prostaglandins produced (ng ml^{-1}) E_2	Prostaglandins produced (ng ml^{-1}) $F_{2\alpha}$
Control		10	$0.28 \pm 0\ 05$	$0\ 50 \pm 0{\cdot}05$
TMMP	0.1	3	$0\ 44 \pm 0.02$*	0.84 ± 0.08*
retinoic	0.5	3	0.58 ± 0.06*	1.40 ± 0.10*
acid	2 0	3	0.62 ± 0.03*	$1.77 \pm 0\ 25$*
cis retinoic	0 5	3	$0.42 \pm 0\ 10$*	0.75 ± 0.05†
acid	2.0	3	0.92 ± 0.05*	1.03 ± 0.18*
trans retinoic acid	2.0	3	0.62 ± 0.04*	1.27 ± 0.06*
Retinyl acetate	2.0	3	0.86 ± 0.07*	0.99 ± 0.06*
Retinol	2 0	3	$0\ 76 \pm 0.04$*	1.00 ± 0.20*
Retinal	2.0	3	0.53 ± 0.04*	0.86 ± 0.07*
Retinyl palmitate	2.0	3	0.29 ± 0.02‡	$0\ 57 \pm 0\ 05$‡

MDCK cells were incubated with the retinoids for 22 h. Each retinoid at the dose used does not cross-react with anti-prostaglandin antisera Experiments with retinoids were carried out several times with qualitatively similar results The values are the mean ± s.d

* $P < 0.001$.

† $P < 0.01$.

‡ Not significant.

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Table 2 Effect of retinoids and TPA on prostaglandin production and the release of radioactivity from ^{3}H-arachidonic acid-labelled MDCK cells

Treatment	Radioactivity (c.p.m. × 10^{-3} per dish)	Prostaglandins produced (ng ml^{-1}) PGE$_2$	PGF$_{2\alpha}$	6-keto-PGF$_{1\alpha}$
None	19.9 ± 1.0	0.11 ± 0.04	0.66 ± 0.09	0.47 ± 0.12
+TPA	93.3 ± 6 4*	2.47 ± 0.35*	6.50 ± 0.66*	9.03 ± 0.93*
cis retinoic acid	36.6 ± 0.6*	0.38 ± 0 05†	1.15 ± 0.05†	2.13 ± 0.40*
+TPA	101.6 ± 1 7*	2.32 ± 0.21*	4.83 ± 0.59*	10.10 ± 1.77*
TMMP retinoic acid	43.2 ± 1.3*	0.64 ± 0.07*	2 60 ± 0.35*	4.23 ± 0.75*
+TPA	172.3 ± 1.4*	5 20 ± 0.58*†	12.67 ± 0.58*	22.67 ± 0.58*

MDCK cells were treated for 22 h with TPA (0.0005 μg ml^{-1}, Consolidated Midland), *cis* retinoic acid (2 μg ml^{-1}, Roche), TMMP retinoic acid (2 μg ml^{-1}, Roche) or their mixture. Prostaglandins produced were analysed by radioimmunoassay. Production of 6-keto-PGF$_{1\alpha}$ by MDCK cells was verified by high pressure liquid chromatographic analyses[21] The values are the mean ± s.d. of triplicate dishes. The differences between 'none' and *cis* retinoic acid or TMMP retinoic acid are statistically significant. The differences between 'none' and TPA; *cis* retinoic acid and *cis* retinoic acid plus TPA; and TMMP retinoic acid and TMMP retinoic acid plus TPA are statistically significant ($P<0.001$). The differences between TPA and *cis* retinoic acid plus TPA are not statistically significant. Those between TMMP retinoic acid plus TPA and TPA or *cis* retinoic acid plus TPA are statistically significant.

* $P<0.001$. † $P<0.005$.

(MDCK) cells. Treatment of the cells with a phorbol diester and a trimethylmethoxyphenyl analogue of retinoic acid led to a synergistic stimulation of lipid deacylation and prostaglandin production.

The MDCK cells were grown in Eagle's minimal essential medium containing 2 mM L-glutamate and supplemented with 10% (v/v) fetal bovine serum and penicillin–streptomycin. Cells were plated at 2×10^5 cells per dish and incubated in non-serum-supplemented medium with and without increments of retinoids and/or the phorbol diester for 22 h. Triplicate dishes were used for each reagent. Medium was assayed serologically for PGE$_2$, PGF$_{2\alpha}$, and 6-keto-PGF$_{1\alpha}$, with anti-PGE$_2$, anti-PGF$_{2\alpha}$ and anti-6-keto-PGF$_{1\alpha}$ antisera (the anti-PGE$_2$ antiserum reacts 0.01% with PGF$_{2\alpha}$ and the anti-PGF$_{2\alpha}$ antiserum reacts 0.1% with PGE$_2$, but the antisera do not identify the prostaglandins as either monoenoic or dienoic). The MDCK cells were labelled with ^{3}H-arachidonate as described previously[4]. They were extracted with 2:1 chloroform:methanol and the extract chromatographed on thin-layer silica gel G (Merck). Analysis of the radioactive materials showed that 69% of the radioactivity was associated with phospholipids, 15.0% with triglycerides, 11% with unidentified materials, 3.4% with prostaglandins and 1.4% with free arachidonic acid.

MDCK cells incubated in the presence of the trimethylmethoxyphenyl (TMMP) analogue of retinoic acid produced increased levels of PGE$_2$ and PGF$_{2\alpha}$. This stimulation was observed with as little as 0.1 μg ml^{-1} (2.7×10^{-7} M) TMMP retinoic acid (Table 1). At the concentrations of retinoids used, the number of cells adherent to the culture dish was not affected, although higher levels of some of the retinoids (~3×10^{-5} M), especially *cis* and *trans* retinoic acid and retinyl acetate, did detach cells from the dish. The stimulating activities of the different retinoids varied (Table 1). All the retinoids stimulated prostaglandin production, except retinyl palmitate, which, at levels >5.0 μg ml^{-1}, inhibited prostaglandin production. However, this inhibition could be accounted for by its content of the phenolic antioxidant, butylated hydroxyanisole, used as preservative, which inhibits prostaglandin production (K.O. and L.L., unpublished data).

The synthesis of the prostaglandins by MDCK cells is limited by the availability of the precursor polyunsaturated fatty acids[5]. Stimulation of deacylation of the cellular lipids was demonstrated by the release of radioactive materials from ^{3}H-arachidonic acid-labelled cells treated with *cis* retinoic acid or TMMP retinoic acid (2.0 μg ml^{-1}, Table 2). Levels of PGE$_2$, PGF$_{2\alpha}$ and the prostacyclin non-enzymatic product, 6-keto-PGF$_{1\alpha}$, were also increased. The tumour-promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), was even more effective than the retinoids at stimulating release of radioactive materials and enhancing prostaglandin and prostacyclin production. The stimulating effects of both TPA and TMMP retinoic acid were more than additive; they acted in a synergistic manner. The effects of *cis* retinoic acid and TPA were not synergistic; in fact, their combined effect was slightly less than additive.

Studies of the effects of vitamin A and its analogues on carcinogenesis have led to conflicting results. On the one hand, they modify skin tumour promotion and prevent epithelial cancers[6–10]. Topical applications of retinoids inhibit skin papilloma formation and the tumour-promoting phorbol diester induction of ornithine decarboxylase activity[11]. However, enhancement of the effects of the chemical carcinogens, similar to that of the tumour-promoting phorbol diesters, has also been described[12–15]. With respect to cultured cells, pathways leading to membrane glycosylation may involve vitamin A as a lipid intermediate[16,17] and vitamin A can alter surface membrane glycolipids and glycoproteins[18]. Retinoic acid inhibits the co-mitogenic action of TPA in bovine lymphocytes[19], and retinoic acid and retinoids, at low concentrations, stimulate plasminogen-activator synthesis in chick embryo fibroblasts; at suboptimal levels of TPA, the effects of retinoic acid and TPA are synergistic[20]. Clearly, the biological properties of the retinoids are manifold and their promoting or anticarcinogenic properties may depend on the type of membrane change induced, which probably in turn depends on the cell and the particular synthetic retinoid used. We suggest that the use of vitamin A and some of its analogues to prevent chemical carcinogenesis is premature; they may enhance, not inhibit tumour formation.

Unless indicated, the retinoids were from Sigma.

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Uncoupled synthesis of histones and DNA during Friend cell differentiation

ERYTHROLEUKAEMIC mouse spleen cells (Friend cells) can undergo erythropoietic differentiation *in vitro* when cultured in the presence of a variety of different inducers[1–6]. Untreated Friend cells are arrested at a proerythroblast-like stage of development in the erythropoietic lineage[7] and contain little or no globin mRNAs and no haemoglobin. In response to an inducer the cells start transcribing the globin genes, accumulate globin mRNAs and haemoglobin in the cytoplasm[3,8] and express morphological and biochemical changes which parallel changes observed during normal *in vivo* erythropoiesis in the mouse. Dimethyl sulphoxide-induced Friend cells (strain 745 A) show a transient block in the initiation of DNA synthesis which precedes detectable expressions of the differentiated functions[9]. We report here a G1 phase prolongation in another Friend cell line using another inducer (*n*-butyrate). We have also characterised the induction process in respect of the capacity of the cells to synthesise and transport proteins to the nucleus. The rate of synthesis of total nuclear proteins decreases steadily but the histones show a reproducible pattern of an initial increase in this parameter at a time when DNA synthesis is blocked, indicating that histone synthesis becomes independent of DNA replication during Friend cell differentiation.

A series of cultures was prepared starting from a single culture of F4N cells[10] in exponential growth. *n*-Butyrate was added and at various times thereafter cells from one culture were collected for determination of cell density, incorporation by whole cells of [methyl-^{3}H]thymidine into acid-precipitable material and appearance of radioactive label in total nuclear proteins and in histones after labelling of cells with ^{3}H-lysine and ^{3}H-arginine. The results of such an experiment are shown in Fig. 1. In Fig. 1*a* cellular DNA synthesis after addition of *n*-butyrate is related to the percentage of haemoglobin-containing, benzidine-reactive (B$^+$) cells[6]. For 4 h after addition of inducer the rate of DNA synthesis was unchanged but between 4 and 8 h after addition DNA synthesis decreased to half the original rate. This low-level DNA synthesis continued until 12 h after addition of inducer, when the rate of DNA synthesis increased to levels characteristic for exponentially growing cells: this period was followed by a final cessation of DNA synthesis. A significant increase in the number of haemoglobin-producing cells was observed only at about 20 h after the onset of *n*-butyrate treatment. The DNA synthesis curve and cytofluorometric determination of the DNA content of single cells suggest that an early effect of the induction process is a transient prolongation of the G1 period of the cell cycle resulting in an extensive depletion of S-phase cells[11]. A similar prolongation has been described for dimethylsulphoxide-induced Friend 745A cells[9] although the time scale for the changes is different. *n*-Butyrate acts much more rapidly than dimethylsulphoxide in inducing the expression of the differentiated function as monitored by the appearance of B$^+$ cells. In accordance with this the prolongation of the G1 phase in our system is expressed both earlier and is of shorter duration than the prolongation observed with dimethylsulphoxide[9]. The G1 phase prolongation seems to be an inherent feature of the commitment process and might be necessary for the synthesis of specific regulatory substances required during the following S phase. This first S phase in the presence of the inducer seems to be unusual in some respects: the activity of the nuclear enzyme poly (ADP-ribose) polymerase is two- to fourfold greater, and

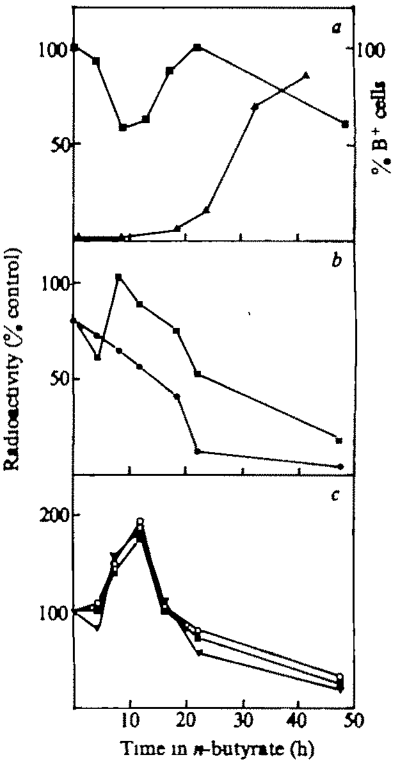

Fig. 1 Rate of DNA synthesis and appearance of newly synthesised proteins in the nuclei of *n*-butyrate-treated F4N cells. Ostertag's erythroleukaemic mouse spleen cell line F4N was used[2] and cells were kept in culture conditions described previously[10]. For induction of the erythropoietic differentiation 1 mM *n*-butyrate was added to culture medium supporting exponentially growing cells (2–3 × 10^5 cells ml^{-1}) and cells were kept for various periods in the presence of the inducer. *a*, Incorporation of ^{3}H-thymidine into DNA (c.p.m. per 10^3 cells) (■) and appearance of benzidine reactive (B$^+$) cells (▲). For the measurement of DNA synthesis 1-ml aliquots of cells that had been kept in *n*-butyrate for the time shown on the abscissa were incubated with 3 μCi [methyl-^{3}H]thymidine (50 Ci mmol^{-1}) for 20 min at 37 °C. Trichloroacetic acid (TCA) precipitable material was determined by liquid scintillation counting. The percentage of benzidine reactive cells was determined using the suspension staining technique[10]. Between 200 and 300 cells were counted for each determination *b*, The appearance of newly synthesised proteins in the nuclear fraction of *n*-butyrate-treated cells (total nuclear proteins, ●, histones, ◆) expressed as per cent of untreated, exponentially growing cells was determined as follows. In sterile conditions 3–5 × 10^7 cells were collected, washed once with 5 ml lysine- and arginine-free culture medium, resuspended in 3 ml of the same medium which was substituted with 50 μCi ^{3}H-lysine (74 Ci mmol^{-1}) and 50 μCi ^{3}H-arginine (18 Ci mmol^{-1}). Incubation was at 37 °C for 150 min and the reaction stopped by chilling on ice; the cells were collected and washed once with 5 ml cold phosphate-buffered saline (PBS, 0.14 M NaCl, 2.7 mM KCl, 1.47 mM KH_2PO_4, 8.1 mM Na_2HPO_4), containing 1 mM phenylmethylsulphonylfluoride (PMSF). All subsequent buffers and solutions contained 1 mM PMSF to prevent proteolytic degradation. The cells were resuspended in PBS, PMSF and lysed with 0.15% Kyro EOB (Procter and Gamble). After 10 min at 4 °C no intact cells were detected as judged by Trypan blue staining. The released nuclei were pelleted at 1,000*g* for 15 min and suspended in 20 volumes of 0.15 NaCl, 50 mM Tris-HCl, *p*H 7.5, 1 mM PMSF. The proteins in this nuclear suspension were referred to as total nuclear proteins. The nuclei were pelleted again and washed twice more with 20 volumes of the same solution. At this stage the nuclei could be stored frozen at −20 °C for several days before histone extraction. Histones were extracted from the washed nuclei using 0.125 M H_2SO_4, 1 mM PMSF. Nuclei were suspended in 10 volumes of this solution with a Vortex mixer and left for 1 h in an ice bath with occasional stirring. The suspension was centrifuged for 20 min at 10,000*g* at 4 °C, the supernatant was taken away and the pellet was extracted in the same way once again. The two supernatants were combined and referred to as total histones. Determination of the incorporation of the radioactive amino acids into total nuclear proteins and into histones: aliquots of the nuclear suspension in 0.15 M NaCl, 50 mM Tris-HCl *p*H 7.5, 1 mM PMSF and of the acid extracted histones were precipitated with 20% TCA and were left for 30 min on ice. The protein precipitate was collected on Millipore filters and washed four times with 5 ml cold 12% TCA. The radioactivity on the filters was determined by liquid scintillation counting. *c*, Incorporation of ^{3}H-lysine and ^{3}H-arginine into histone H1 (▼); histones H3, H2a, H2b (■); and histone H4 (○). The histone classes were separated by SDS-polyacrylamide electrophoresis[15], the Coomassie blue stained protein bands cut out, the proteins extracted from the gel slices with 0.5% SDS and the radioactivity in the extracts determined by liquid scintillation counting.

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the extent of release of Friend virus sixfold greater than the levels observed in cells in exponential growth phase[12].

General protein synthesis gradually increases during the cell cycle, with the notable exception of histones. Synthesis of histones is restricted to the S phase of the cell cycle and is correlated with the synthesis of DNA[13] suggesting that the chromosome as a whole and not just its genetic material is reproduced during S phase. As *n*-butyrate treatment changes the course of the cell cycle we studied the labelling and appearance of histones in the nuclei during the early stages of the induction period. Figure 1*b* shows synthesis and appearance of histones and of total nuclear proteins in nuclei of *n*-butyrate treated cells. Radioactive labelling was done with ^{3}H-arginine and ^{3}H-lysine for 150 min in conditions where uptake of radioactivity was linear for more than 4 h. The rate of protein synthesis and rate of appearance of proteins in the nucleus steadily decreased during the 48-h period studied. This is similar to the early decrease in total cellular protein synthesis which has been recently reported for Friend cells in which haemoglobin synthesis has been induced by actinomycin D (ref. 14). In contrast to this decrease is the increasing level of newly synthesised histones in the nuclei: an initial period of 4 h with no change is followed by a pronounced increase in the amount of newly synthesised histones to a level two times greater than that in exponentially growing cells. This increase takes place concurrently with a decrease in the rate of DNA synthesis. These data suggest that an uncoupling of histone synthesis and DNA synthesis occurs before haemoglobin is produced. This dissociation represents an early effect after the addition of the inducer and leads to a higher rate of synthesis and transport of histones to the nucleus than in a state of exponential cell proliferation.

To establish whether all histone fractions respond in parallel or whether there is a differential effect in the different histone classes, labelled histones were separated by electrophoresis on SDS-polyacrylamide gels[15]. Histone bands located by Coomassie blue staining were excised and their radioactivity content determined. Separation of histones H3, H2a and H2b was not satisfactory and the three histones were treated as one fraction. The rate of accumulation of newly synthesised histones H1, H4 and (H3+H2a+H2b) is shown in Fig. 1*c*. The rate of appearance of the newly synthesised histones in nuclei is similar for all classes, indicating that the synthesis of nucleosomal histones and the synthesis of histones binding to DNA in the internucleosomal region are regulated coordinately and dissociated from DNA synthesis. This special mode of histone synthesis seems to occur in parallel with hyperacetylation of histones which has been recently described in *n*-butyrate-induced Friend cells[16] and which seems to be due to inhibition of histone deacetylation[17].

The changes in DNA and histone synthesis described here may reflect some intrinsic features of the commitment event. A prolonged G1 phase is characterised by an increase in the rate of appearance of newly synthesised histones in the nuclei. This increase is seen in all histone classes (including $H1_0$ (ref. 18) but not in total nuclear proteins which show a steady decrease in their rate of synthesis. Prolongation of the G1 phase may facilitate the synthesis and modification of regulatory molecules required for the S phase during which reprogramming towards the expression of the differentiated function may take place.

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Cell specificity in metabolic activation of aflatoxin B_1 and benzo(*a*)pyrene to mutagens for mammalian cells

ENVIRONMENTAL chemical carcinogens are usually nonreactive and have to be enzymatically activated before they can manifest their biological effects[1–6]. Many cell types, including those used for mutagenesis studies, are not capable of activating such procarcinogens; mutagenesis in these cells has been studied by the addition of an enzymatically active liver subcellular fraction[7–9]. However, these liver subcellular fractions readily activate a wide spectrum of compounds into potent mutagens including those which are not carcinogenic to the liver. In addition subcellular preparations differ from intact cells in the profile of the metabolites[10,11] and DNA adducts[12,13] formed after metabolism of various potent carcinogens such as aflatoxin B_1 (AF), benzo(*a*)pyrene (BP), and 7,12-dimethylbenz(*a*)anthracene. These results suggest that the use of subcellular fractions does not truly simulate the *in vivo* situation and

Table 1 Binding of BP to the DNA of fibroblasts and liver cells

	BP binding (ng bound BP per mg DNA) after treatment with: 1.0 μg ml^{-1}	0.1 μg ml^{-1}
Fibroblasts	8.9 ± 3.6	2.0 ± 0.8
Liver cells	3.2 ± 0.4	1.1 ± 0.2

Binding of BP to cellular DNA was determined 24 h after treatment with BP of 10^7 fibroblasts and 7×10^6 liver cells grown in 10 ml of medium per 100-mm Petri dish; 5–7 Petri dishes were used per data point. For isolation of the DNA-BP products, the treated cells were trypsinised, washed extensively with phosphate-buffered saline (*p*H 7.4), and then lysed in buffer containing 10 mM Tris-EDTA, 100 mM Nacl, and 1% SDS (*p*H 7.4). Lysates were extracted twice with an equal volume of distilled phenol, once with an equal volume of 1 part phenol to 1 part chloroform-isoamyl alcohol (v/v 24:1), and a further three times with chloroform-isoamyl alcohol (24 : 1), resulting in a clear interface. The nucleic acids were precipitated from the aqueous phase with 2.5 volumes of absolute ethanol and centrifuged at 1,000*g* for 10 min at 4° C. The precipitate was dissolved in 0.1 SSC (0.015 M NaCl–0.0015 M sodium citrate), and the RNA was digested with ribonuclease A 50 U ml^{-1} for 40 min at 37° C. Phenol and chloroform extractions were repeated as described above, and the DNA was precipitated with 2.5 volumes of absolute ethanol in the presence of 0.1 M NaCl. The precipitate was dissolved in H_2O, and the DNA was determined quantitatively by absorbance at 260 μm. The ratio A_{260} to A_{280} was 1.95 ± 0.05. The BP bound to DNA was determined by dissolving an aliquot in scintillation solution and counting the samples in a Tri-Carb liquid scintillation spectrometer. Both ^{14}C-labelled BP (62.3 mCi mmol^{-1}) and ^{3}H-labelled BP (4.4 Ci mmol^{-1}) were used in the binding studies. Values means ±s.d. derived from four independent experiments, two with each type of label.

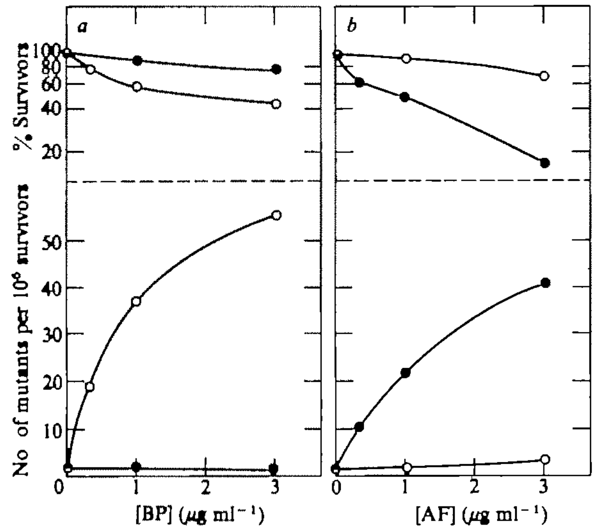

Fig. 1 Fibroblast- and liver cell-mediated mutagenesis by the carcinogens BP (*a*) and AF (*b*). Mutation to ouabain resistance was determined in Chinese hamster V79 cells after co-cultivation with either liver cells or fibroblasts derived from Sprague–Dawley rats. Liver cells were obtained after *in vivo* collagenase perfusion of a liver from a 6- to 10-week-old male rat[18,23]. V79 cells were seeded at 10^5 cells per 25-cm^2 T-flask in 4 ml Williams medium containing 10% fetal calf serum supplemented with 2 mM glutamine, pencillin (100 U ml^{-1}), and streptomycin (100 μg ml^{-1}) at 37° C with an atmosphere of 5% CO_2 in air. After 18 h either 2×10^6 rat embryo fibroblasts (lethally irradiated with 5,000 R) or 10^7 primary rat hepatocytes (80–90% viable by Trypan blue dye exclusion and plating efficiency of 20%) were seeded on the V79 cells. After 3 h the medium was changed and the carcinogens were added in 8 ml of fresh medium. The cells were dispersed 48 h later with 0.05% trypsin and 0.02% EDTA, and the V79 cells, which could be distinguished in the haemocytometer from the fibroblasts and liver cells, were seeded at 200 cells per 50-mm Petri dish in 4 ml medium to determine cloning efficiency and at 10^5 cells per 50-mm Petri dish to determine the number of ouabain-resistant colonies. Ouabain was added 48 h later in 1 ml medium to a final concentration of 1 mM. Six dishes were stained with Giemsa 6–7 d after cell seeding to determine cloning efficiency, and 20 dishes were stained after 14–16 d to determine the number of ouabain-resistant colonies. Data are expressed as the number of ouabain-resistant mutants per 10^6 survivors based on the number of cells seeded for mutant selection and on the cloning efficiency. Data are the results of three separate experiments, and each data point was determined in duplicate. The number of mutants after induction with BP in the fibroblast-mediated assay and AF in the liver-cell-mediated assay varied up to about 25% in the three independent experiments. In all other cases the number of mutants in different experiments varied up to 40%. The cloning efficiency of V79 cells co-cultivated with liver or fibroblast cells was approximately 60%. Cytotoxicity is expressed as % survivors based on a comparison between the cloning efficiency of V79 cells co-cultivated in the absence of carcinogen and that in the presence of the carcinogen. After co-cultivation the number of V79 cells per T flask ranged from 1.2 to 2.8×10^6 cells. The heritability of ouabain resistance was shown with 10 isolated ouabain-resistant colonies, all of which remained resistant to the drug after 1 month in its absence. ●, Liver cells; ○, fibroblasts.

that it may be advantageous to use intact cells for metabolic activation of chemical carcinogens. Therefore, studies of carcinogen metabolism and mutagenic activity in cultured cells derived from different tissues should more truly reflect the *in vivo* situation and should also provide insight into the problem of tissue specificity in chemical carcinogenesis. Metabolic activation by intact cells of different carcinogens into intermediates which are mutagenic to mammalian cells has been reported[4,14–19]. This report compares the abilities of two different cell types, fibroblasts and liver cells derived from rats, to metabolise two carcinogens (BP, a potent skin and lung carcinogen which can also produce fibrosarcomas[20], and AF, a potent liver carcinogen[21]) to water-soluble products, to intermediates which bind to cellular DNA, and to intermediates which are mutagenic to Chinese hamster V79 cells. Ouabain resistance was used as the genetic marker in V79 cells[14,22].

Direct mutagenicity (that not requiring further metabolism of the carcinogens) was determined by testing the induction of ouabain resistance by AF and BP in V79 cells which do not metabolise these carcinogens. The results indicate that neither BP nor AF induced ouabain resistance in V79 cells at concentrations up to 3 μg ml^{-1}. Co-cultivation of the V79 cells with rat fibroblasts which are capable of metabolising BP (fibroblast-mediated assay) or with rat liver cells which metabolise AF (hepatocyte-mediated assay) in the absence of the carcinogens did not alter the mutation frequency. BP in the fibroblast-mediated assay caused 17-, 34-, and 55-fold higher mutation frequencies than the control at 0.3, 1.0 and 3.0 μg ml^{-1}, respectively (Fig. 1*a*), while in the hepatocyte-mediated assay no significant increase was observed in the mutation frequencies at BP doses up to 3 μg ml^{-1}. Treatment of the V79 cells with AF at 0.3, 1.0 or 3.0 μg ml^{-1} in the hepatocyte-mediated assay resulted in 10-, 20- and 40-fold higher mutation frequencies than the control (Fig. 1*b*). In the fibroblast-mediated assay AF caused only a twofold increase at a dose as high as 3 μg ml^{-1}.

The differences observed in the mutagenic activities of BP and AF in the hepatocyte- and fibroblast-mediated assays suggest that these two carcinogens are metabolised differently in each of the two cell types. Therefore the extent of BP and AF metabolism to water-soluble metabolites[11,24] by hepatocytes and fibroblasts was compared. The results indicate that liver cells metabolise both BP and AF to water-soluble products, while fibroblasts metabolise BP but not AF (Figs 2, 3). For both cell types the metabolism of BP to water-soluble products was proportional to the cell number (Fig. 2*a*), the dose of the hydrocarbon (Fig. 2*c*), and the time of incubation up to 48 h (Fig. 2*b*). After 48 h, BP metabolism by liver cells reached a plateau, probably due to cell degeneration, while in the fibroblasts BP metabolism continued for an additional 24 h. The metabolism of AF by liver cells was also proportional to the cell number, the dose of the carcinogen, and the time up to 24 h (Fig. 2*a–c*). The liver cells showed only a limited increase in AF metabolism after 24 h, presumably due to the cytotoxic effects of AF metabolites and degeneration of the hepatocytes in culture. The fibroblasts did not metabolise AF (Fig. 3*a–c*), and this can explain the absence of AF mutagenicity in the fibroblast-mediated assay.

Therefore, even though on a per-cell basis, liver cells were able to metabolise BP to water-soluble products four to five times as effectively as fibroblasts (Fig. 2*a–c*), in the hepatocyte-mediated assay BP was not mutagenic (Fig. 1*a*). These findings raised the question of production and/or release of mutagenic BP metabolites by the liver cells. To estimate the relative production of reactive BP intermediates by the two cell types, the binding of BP metabolites to DNA of both cell types was measured. After 24 h exposure to BP at 1 μg ml^{-1}, approximately three times as much BP was bound to the DNA of the fibroblasts as was bound to the DNA of the liver cells (Table 1). Thus even though the liver cells metabolised four to five times as much BP at 1 μg ml^{-1} to water-soluble products, they had substantially less DNA-bound BP products than the fibroblasts. These findings indicate either that liver cells metabolise BP to less reactive intermediates as compared with fibroblasts, or that if such reactive metabolites are formed they are rapidly detoxified. It has been reported that the degree of covalent binding of BP metabolites to DNA is regulated by their relative rate of production and their rate of detoxification[25].

The mutagenic activity of BP in the fibroblast-mediated assay but not in the hepatocyte-mediated assay and the inverse situation with AF are in agreement with the *in vivo* activities of these two carcinogens. However, 'S-9' subcellular fractions from liver tissue have been reported to activate both carcinogens to

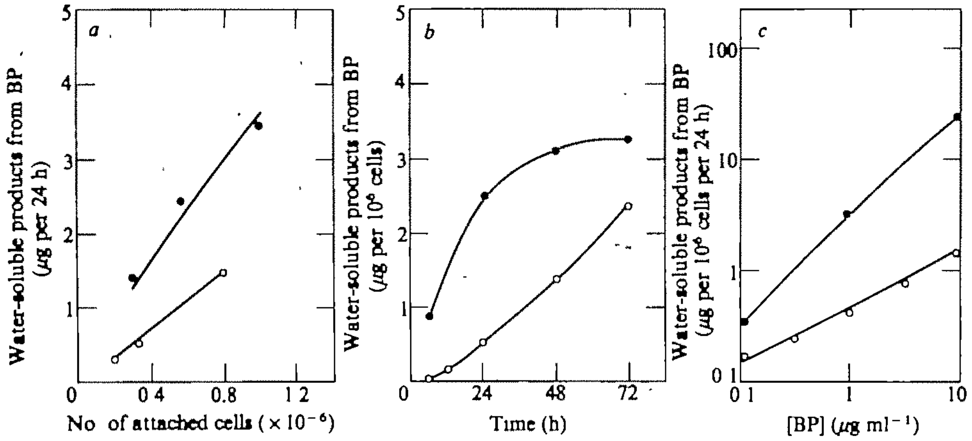

Fig. 2 Metabolism of BP by liver cells and fibroblasts. Liver cells were isolated after *in vivo* collagenase perfusion of the liver of a 6- to 10-week-old male rat, and the cells (viability, 80–90%) were seeded into 25-cm^2 T flasks in 5 ml medium. The plating efficiency was about 20%. Lethally irradiated (5,000 R) rat embryo fibroblasts were seeded in 5 ml of medium into 25-cm^2 T flasks. The plating efficiency of the fibroblasts was about 80%. At 3 h after seeding the medium was changed and 5 ml medium containing the ^{14}C-AF or ^{14}C-BP was added. The reaction was terminated by addition of 5 ml of a 1% SDS solution to each T flask[24]. The contents of the flask were then mixed with a pipette to ensure that all cells lysed, and 5 ml was removed and mixed with nine volumes of chloroform–methanol (2:1). The solution was vigorously mixed and then centrifuged. Duplicate 0.5-ml aliquots of the aqueous (upper) phase were removed and added to 10 ml Aquasol for counting in a Beckman Model B scintillation counter. Total recoveries were 90–100% as determined by summing radioactivities in the aqueous and organic phases. In the absence of cells, 0.3–0.6% of the ^{14}C-BP and 3.0–4.5% of the ^{14}C-AF were soluble in the aqueous phase; this was considered as background. All experiments were run with three dishes per point, and each experiment was repeated 2-3 times. The data presented are the mean values, and the variance between different experiments was up to 15%. Liver cell numbers were determined by counting five different fields of the T flask with a grid. Stock solution of the medium containing a known amount of the radioactive carcinogen AF (2–3 × 10^4 c.p.m. ml^{-1}; [G-^{14}C]AF) or BP (4-5 × 10^4 c.p.m. ml^{-1}, [7,10-^{14}C]BP) with the desired quantities of unlabelled BP or AF was added to each culture. Thus the total radioactivity in an individual experiment remained constant, but the specific activity decreased as the dose increased. ●, Liver cells; ○, fibroblasts.

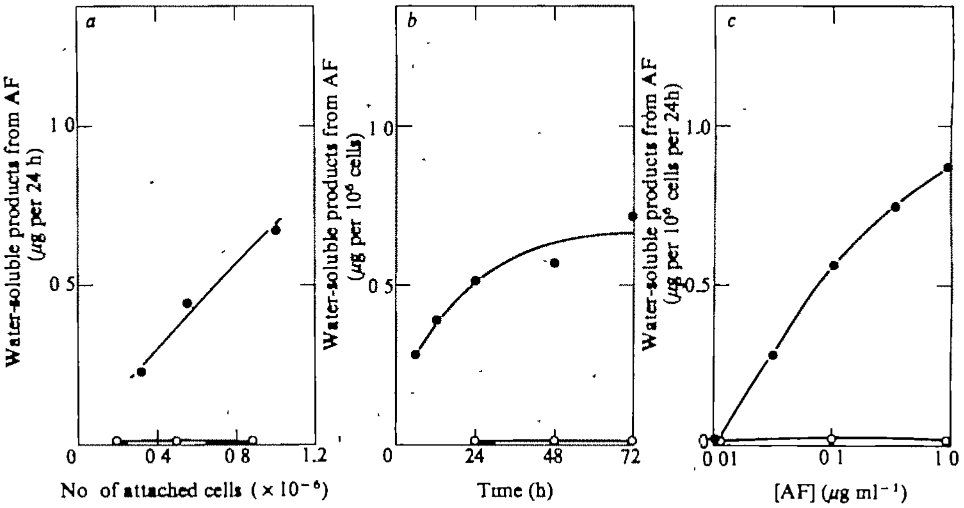

Fig. 3 Metabolism of AF by liver cells and fibroblasts. For details see Fig. 2. ●, Liver cells; ○, fibroblasts.

intermediates which are mutagenic for both *Salmonella typhimurium*[7] and V79 cells[8]. These differences in the metabolic activation may result from the disruption in the liver subcellular preparations of the normal balance of pathways by which BP and AF are activated and detoxified in the intact liver.

Our results indicate that a cell type specificity in chemical carcinogenesis can be investigated by the cell-mediated assay. Furthermore, while the metabolism of procarcinogens is required to manifest *in vitro* mutagenic activity, overall metabolism alone is not sufficient to predict the activity of a carcinogen in a given cell type. The discrepancy between the metabolic activation of the same chemical carcinogens by subcellular fractions as opposed to intact cells and the loss of cell specificity by the former lead us to suggest that the cell-mediated assay may provide a more useful means of screening environmental chemicals and may also be used to study tissue specificity of chemical carcinogens *in vitro*.

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Volatile nitrosamines in normal human faeces

NITROSAMINES are a major class of chemical carcinogens which lead to a wide variety of tumours in many animals[1]. They may be formed *in vitro* by nitrosation of primary[2], secondary[3] or tertiary amines[4] and are found in air[5], water[6], foods[7] and consumer products[8]. They may also be formed *in vivo* in the stomach or small intestine of experimental animals following the concurrent administration of nitrite and precursor amines[9–12]. Here we show that several volatile nitrosamines are present in normal human faeces and suggest that they have been produced endogenously in the lower gastrointestinal tract.

Previously, we had used freeze-dried faecal samples for studies of the mutagenic compounds (presumptive carcinogens) in faeces[13]. However, this method does not test for volatile compounds of possible interest. Hence, the freeze-drier condensate was extracted with dichloromethane and this extract was injected on either a gas–liquid chromatograph (GLC) or a high-pressure liquid chromatograph (HPLC), connected to a thermal energy analyser (TEA). Several peaks were observed (Fig. 1) and were attributed to nitroso compounds, as the TEA detector is highly specific for NO-containing compounds. By comparison with standards, the five major peaks were tentatively identified as due to *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP) and *N*-nitrosomorpholine (NMOR), the amounts present in a typical condensate being, respectively, 0.04, 0.10, 0.08, 0.08 and 0.02 μg per l.

The assignment of the TEA peak from the faecal condensate was confirmed by four methods. First, the estimates of the five nitrosamines obtained by either GLC or HPLC were found to agree quantitatively. Second, the isolated fraction corresponding to the peak for NDMA on HPLC was found to give only one peak on GLC–TEA, corresponding to the position of NDMA. The same was true for NDEA, NDBA, NPIP and NMOR.

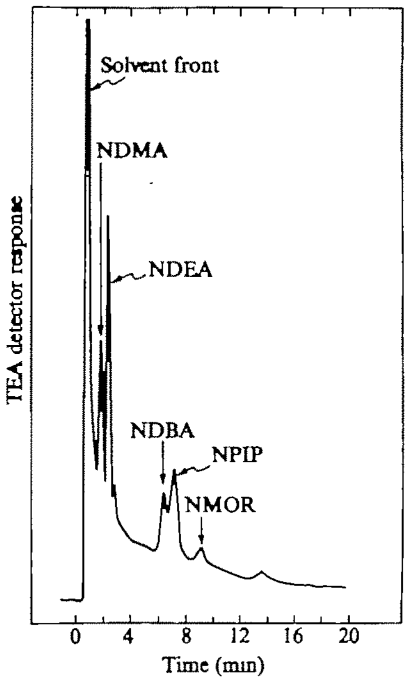

Fig. 1 GLC for a 5-μl DCM extract of the condensate from the freeze drier. 14 feet of stainless steel tubing (outer diameter 1/8th inch), packed with 10% Carbowax 20M on Chromosorb W, 80–100 mesh was used for a single-column isothermal gas chromatograph. The carrier gas was argon at a flow rate of 30 ml min^{-1} and the column oven temperature was 150 °C. A model TEA-502 thermal energy analyser (TEA, Thermo Electron) was used as the detector TEA attenuation was ×8. The last peak on the chromatogram is unidentified.

Third, when the dichloromethane extract of the condensate was treated with HBr in glacial acetic acid it no longer gave any peaks on the TEA detector. The same was true when the extract was transferred into aqueous acid solution and exposed to ultraviolet light. As both these treatments are known to cleave the N–NO bond of nitrosamines[14,15], the peaks seen in Fig. 1 were presumably due to nitrosamines. Fourth, the fractions corresponding to NDBA and NPIP were purified by HPLC after several clean-up procedures[16] and the presence of the compounds in the purified fractions was confirmed by comparing the spectra obtained with mass spectroscopy (GLC–MS) with that obtained with standards for NDBA and NPIP[17]. The finding that faecal distillates contained volatile nitrosamines prompted an extensive investigation of these compounds in normal faeces.

Faecal samples were collected from 12 healthy volunteers at the Ontario Cancer Institute and immediately extracted with

Table 1 Volatile nitrosamines in normal human faeces

Donor	NDMA	NDEA	NDBA	NPYR	NMOR
		Men (μg per kg)*			
A	0.94	6.76	0.87	0.31	0.23
B	0.27	1.76	0.12	ND	0.07
C	0.51	11.32	ND	ND	0.45
D	0.22	1.91	ND	ND	ND
E	1.20	2.16	ND	ND	1.25
F	0.27	0.18	ND	ND	ND
G	1.11	ND	ND	ND	ND
		Women (μg per kg)*			
H	1.47	2.81	ND	0.78	ND
I	1.06	ND	ND	ND	ND
J	1.49	13.3	ND	0.55	0.51
K	ND	ND	ND	ND	ND
L	0.63	ND	ND	ND	ND

ND, not detectable (<0.01 μg per kg); NDMA, nitrosodimethylamine; NDEA, nitrosodiethylamine; NDBA, nitrosodibutylamine; NPYR, nitrosopyrrolidine; NMOR, nitrosomorpholine.

* After correction with recovery efficiency as shown in text.

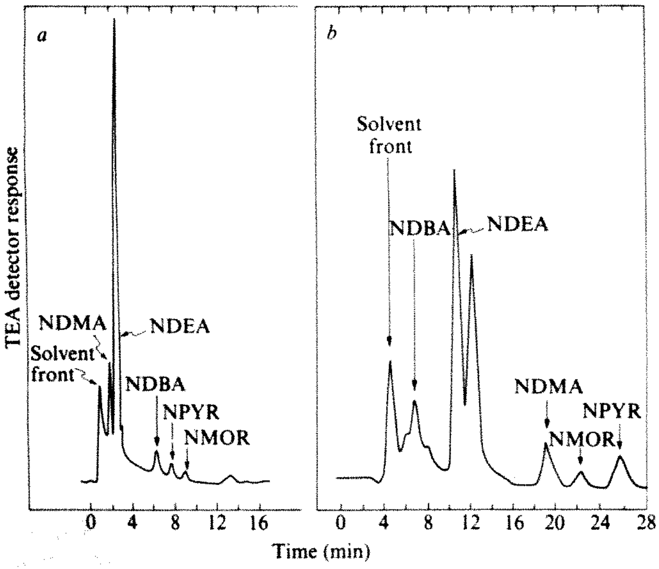

Fig. 2 *a*, GLC for a 10-μl DCM extract of the faecal sample from donor *A*. The operating conditions for GLC–TEA were identical to those described in Fig. 1. *b*, HPLC for a 20-μl DCM extract of the faecal sample from donor *A*. The HPLC–TEA was constructed by connecting a model UK6 injector (Waters Associates) with a model 6000A high-pressure pump solvent delivery system (Waters Associates) and a model TEA-502 thermal energy analyser (Thermo Electron). A μ-porasil column (0.39 cm inner diameter × 30 cm; Waters Associates) was used with a 5% acetone–95% 2, 3, 4-trimethylpentane solvent system at a flow rate of 1 ml min^{-1}. TEA attenuation was ×8. The peak after NDEA on HPLC chromatogram is unidentified.

dichloromethane. The extract was dried with sodium sulphate, concentrated and then distilled from mineral oil to a liquid nitrogen cold trap[18]. The distillate was extracted with dichloromethane and then applied to GLC–TEA or HPLC–TEA in conditions similar to those described by Fine[19]. Control experiments showed that the reagents and chemicals used in the analytical procedure contained no volatile nitrosamines and that the addition of ascorbic acid to the faeces, at a concentration which inhibits nitrosation of amines[20] (1 mg per g of faeces), did not affect the yield. With this method, the recovery of NDMA, NDEA, NDBA, NPIP, NPYR and NMOR at a concentration of 2 μg per kg added to the faeces was found to be 51, 68, 80, 81, 88 and 88%, respectively.

Results of a typical GLC–TEA and HPLC–TEA tracings for the faeces from one volunteer (donor *A*) are shown in Fig. 2. The identification of the peaks as NDMA, NDEA, NDBA, NPYR and NMOR in Fig. 2 was confirmed by the first three methods described above. The results for 12 samples (Table 1) show that most faeces contain NDMA at levels of 0.3–1.5 μg per kg. NDEA was found in higher amounts (0.2–13 μg per kg), with samples from both males and females giving values >10 μg per kg. Two samples had detectable levels of NDBA, in both cases <1 μg per kg. NPYR and NMOR were found in several donors, with detectable levels <2 μg per kg. Many of the levels here are higher than those found in the freeze-dried condensate. Presumably, losses occurred during the freeze-drying period and not all the volatile nitrosamines were retained in the freeze drier.

The source of the nitroso compounds in the faeces has not been established. Perhaps some may arise in food or from nitrosation in the stomach but it seems more likely that they are produced in the lower gastrointestinal tract, as Tannenbaum *et al.*[21] have shown that the nitrite precursor may be present at these sites. It has been suggested that the intestine might be a site for the formation of nitrosamines by bacterial action[22]. The formation of nitrosamines from precursor amines and nitrate at neutral *p*H by some strains of bacteria has been demonstrated by Sander[23] and the formation of *N*-nitrosodimethylamine on incubation of ^{14}C-dimethylamine and sodium nitrite with rat faecal contents has also been reported[24,25]. The levels of volatile nitroso compounds in human faeces are similar to those in some foods[7]. However, the long, continuous exposure to nitroso compounds in faeces could pose a greater hazard than that from many foods. The volatile nitroso compounds present in the faeces will presumably be absorbed and distributed throughout the body. Indeed, the absorption from this site may explain the presence of nitroso compounds in the blood[19] and urine[26] of normal individuals. Although the presence of these nitroso compounds in the human body is surprising, it remains to be determined whether they are responsible for any human cancers.

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Tetrahydropapaveroline induces small granular vesicles in brain dopamine fibres

TETRAHYDROISOQUINOLINES (TIQs) can be formed by the condensation of catecholamines with aldehydes. Their *in vivo* formation has been postulated as a possible mechanism to explain some long-term effects of ethanol on the central nervous system[1,2]. However, there have been several criticisms[3,4] and reviews[5,6] of this hypothesis. Although TIQ formation may not explain all the effects of ethanol, TIQs can influence behaviour[7–9] and they do have transmitter-like properties: they can be formed *in vivo*[10–12]; they can be taken up and released *in vivo* from the adrenergic plexus of the rat iris[13]; they can be taken up into rat brain synaptosome fractions[14]; and TIQs applied exogenously have been localised in peripheral catecholamine tissues with both fluorescence[13] and electron microscopy[15]. Thus, TIQs may accumulate in specific brain regions and influence behaviour by either acting as false transmitters[13] or by displacing endogenous transmitters. Critical to this TIQ hypothesis is the demonstration of TIQ uptake and localisation into central neurone terminals. In the present study, slices of rat caudate nucleus were incubated with TIQs and then examined with the electron microscope. The results provide evidence for both the uptake and localisation of the TIQ, tetrahydropapaveroline (THP), into central dopamine fibres and terminals. This localisation may prove important in understanding the mechanism by which TIQs influence animal behaviour and any possible role for TIQs in chronic alcohol consumption.

We used male Sprague-Dawley rats (250–350 g); razor-blade slices of caudate nucleus were incubated for 30 min at 37 °C with various compounds at 5×10^{-4} M (refs 16–18). Tissues were then fixed with potassium permanganate and examined under the electron microscope[19]. After incubation with THP, small granular vesicles (SGVs) were seen in 5-6% of the bouton population (Fig. 1 and Table 1). These SGV boutons were similar in appearance to those identified by others as containing dopamine[16–18,20].

Two further experiments were carried out to determine whether the THP-induced SGV boutons were dopamine terminals. First, as dopamine is actively accumulated[21,22] but does not normally produce a granular precipitate within dopamine-containing synaptic vesicles[16,23], it might block THP uptake and thus the appearance of SGVs in dopamine terminals. Table 1 shows that caudate nucleus incubated with both THP and a 10-fold concentration of dopamine (5×10^{-3} M) was practically devoid of THP-induced SGV boutons. Second, as striatal dopamine innervation is predominantly ipsilateral[20,24], a unilateral lesion of its source nucleus, the substantia nigra, should cause a decrease in dopamine terminals on the lesioned side[20,24]. Table 1 shows that after unilateral 6-hydroxydopamine lesions[20,25] <1% of the boutons contained THP-induced SGVs as compared to 5–6% in the non-lesioned contralateral hemisphere. Substantia nigra lesions were confirmed by both a decreased glyoxylic acid-induced monoamine fluorescence (ref. 26, without perfusion) in the ipsilateral caudate as compared to the non-lesioned contralateral side and a pronounced contralateral asymmetry 10–15 min after a subcutaneous injection of apomorphine (0.25 mg per kg). Although these results are not conclusive, they indicate that THP can be accumulated in central nerve terminals, that these terminals contain dopamine and that they arise from cell bodies in the substantia nigra. Other monoamine terminal regions might also be expected to accumulate THP.

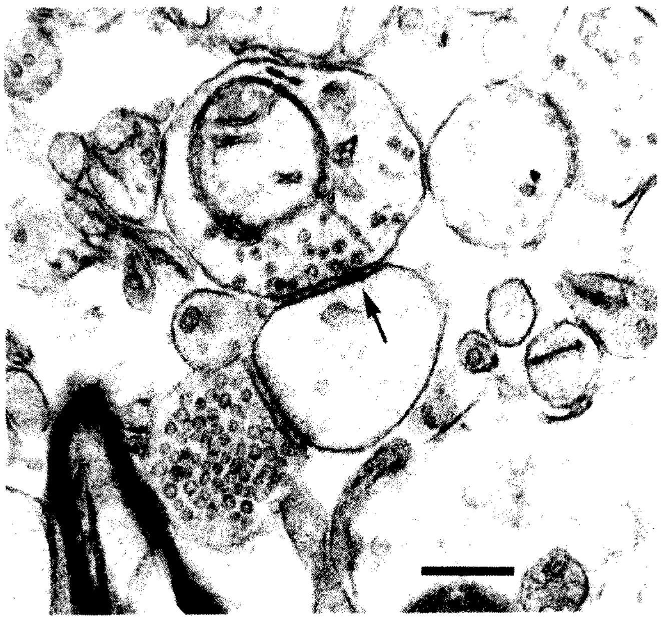
Table 1 Tetrahydropapaveroline-induced small granular vesicle-containing boutons (THP-SGV boutons) in the rat caudate nucleus

	THP-SGV boutons	Total boutons	%THP-SGV boutons
Caudate nucleus from non-lesioned rats			
THP only	497	8,806	5.6
THP + dopamine (5×10^{-3} M)	2	10,391	0.02*
Caudate nucleus after unilateral substantia nigra lesion			
Contralateral to lesion	629	11,761	5.3
Ipsilateral to lesion	48	12,141	0.4*

Slices of rat caudate nucleus were incubated with 5×10^{-4} M THP as outlined in the text. Three unit areas of 6,500 μm^2 from each rat (two rats in each group) were examined.

* $P < 0.001$ (χ^2 test).

Fig. 1 Electron micrograph. The arrow in the dendrite points towards a tetrahydropapaveroline-induced small granular vesicle-containing bouton in the rat caudate nucleus. Note that these granular vesicles are larger in diameter than the agranular vesicles seen in the adjacent bouton. Scale bar, 250 nm.



Our object was in part to identify the THP-induced SGV boutons as either dopamine- or non-dopamine-containing terminals. Thus, the drug concentrations used (5×10^{-4} M) were well above selective uptake levels but were similar to the concentrations used by others to demonstrate dopamine terminals[17,18]. In addition, lower concentrations of THP reveal fewer dopamine terminals[27]. Interestingly, incubation with another dopamine-derived TIQ, salsolinol, and also tetrahydropapaverine, apomorphine and morphine (all at 5×10^{-4} M) failed to produce SGV boutons. Incubation with α-methylnoradrenaline or 5-hydroxydopamine (5×10^{-4} M) revealed SGVs in 10–11% of the striatal bouton population; this is similar to values reported by others[18,20].

Compounds localised within vesicles may or may not produce a granular precipitate after permanganate fixation. The efficiency with which a compound produces a precipitate may be determined by its reactivity towards the permanganate ion, or by its ability to precipitate with manganese dioxide[23]. Thus, the presence of a granular precipitate within vesicles probably indicates uptake, but the absence of precipitate could simply reflect the inefficiency or inability of a compound to sequester the electron-opaque manganese dioxide.

Reports by Myers and co-workers[7–9] have renewed interest in the possible relationship of the TIQs to the physiological processes involved in alcohol addiction. In particular, intraventricular injections of THP were reported to induce rats to consume excess quantities of alcohol voluntarily as compared to control rats[7–9]. It was also reported that another TIQ, salsolinol, and a β-carboline, noreleagnine, caused rats to drink alcohol in excess of controls.

If TIQs are important mediators of the effects of ethanol on the brain, the central catecholamine terminals would provide an opportune target for TIQ formation, storage and release. The present study presents ultrastructural evidence that exogenous TIQ can be accumulated and stored in central dopamine terminals. This cytochemical strategy may prove useful in future efforts to prove or disprove the TIQ hypothesis of alcohol action.

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Primary afferent depolarisation of tooth pulp afferents is not affected by naloxone

RECENT studies (for review see ref. 1) have indicated the existence of endogenous opiate-related peptides and opiate receptors in the dorsal horn of the spinal cord and in the trigeminal nucleus caudalis (the caudal-most component of the trigeminal spinal tract nucleus in the brain stem). Both structures have marginal and substantia gelatinosa layers, receive the terminals of Aδ and C nociceptive afferents, and constitute essential components of spinal or trigeminal nociceptive pathways[2,3]. It has been suggested[1,4–7] that endogenous opiate-related peptides located in presynaptic endings play an important part in nociceptive transmission by inhibiting the presynaptic input to neurones in nociceptive pathways; presynaptic inhibition is thought[8] to result from primary afferent depolarisation (PAD). It has previously been found that jaw-opening reflex activity[9–11] and the responses of trigeminal brain stem neurones[10,11] to noxious orofacial stimuli can be suppressed by stimulation of the periaqueductal grey matter (PGM) and that this descending control is at least partially narcotic-related in the cat, as it can often be reversed by administering the opiate antagonist naloxone. Furthermore, the iontophoretic application of the opiate-related peptide enkephalin also depresses the activity of these neurones[12,13]. If the opiate-related peptides are in fact involved in this suppression at a presynaptic level, then by stimulating PGM and related structures one should be able to produce PAD in nociceptive afferents and then abolish the PAD by naloxone administration. We describe here the first test of this proposition, using the tooth pulp (TP) as the source of nociceptive afferents[14,15]. We have found that these afferents are subject to PAD which, however, cannot be abolished by naloxone.

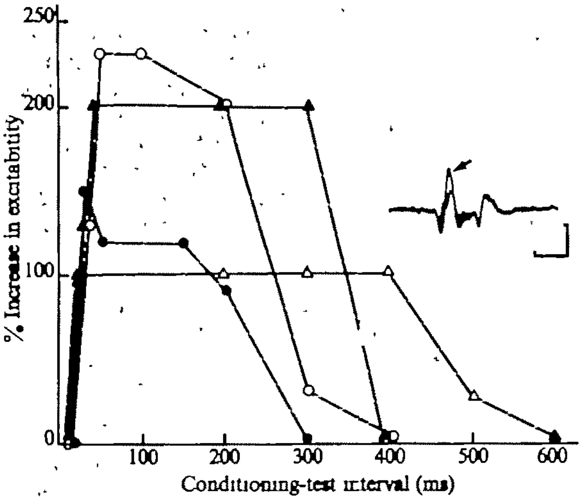

Fig. 1 Increase in antidromic excitability of a single mandibular canine tooth pulp (TP) afferent ending in trigeminal nucleus caudalis produced by conditioning stimulation of PGM (●), NRM (○), maxillary canine TP (▲) and mandibular skin (△). At each conditioning-test interval, the incidence of a spike evoked antidromically by 10 test stimuli to the caudalis was compared with that evoked by a similar number of caudalis stimuli in the presence of a conditioning stimulus. An increase in incidence produced by the conditioning stimulus was expressed as a % increase in antidromic excitability. The insert shows the spike (arrow) evoked antidromically at threshold by one caudalis stimulus, superimposed on a trace where the caudalis stimulus did not elicit the spike although other antidromic activity was evoked Voltage calibration, 0.2 mV; time calibration, 2 ms.

Most of the methods used have been described in detail elsewhere[10,16,17] and will only be briefly described. Thirty cats were anaesthetised with chloralose (60 mg per kg) or decerebrated. Blood pressure, rectal temperature and end-tidal % CO_2 were monitored continuously. In five of these cats, we recorded the activity of single primary afferent units in the trigeminal ganglion that could be orthodromically excited by bipolar stimulation of the ipsilateral canine TP[17] and antidromically activated by brain stem stimulation. In most animals, however, bipolar recordings of TP afferents were actually made from the TP. For such recordings, two drill holes were placed in each of the canine teeth, to a depth at which only a very thin layer of dentine was left, and the TP was carefully exposed by inserting a sharp needle through the thin dentine wall. Bipolar platinum electrodes were implanted in each TP with dental cement. The brain stem was exposed and electrodes located stereotaxically in the trigeminal brain stem nuclei oralis and caudalis, using recordings of maximum field potentials evoked by TP or infraorbital nerve (IO) stimuli, and histological verification. Usually, single-unit activity as well as compound action potentials could be antidromically evoked from TP by stimulation (0.1-ms pulse) of these brain stem nuclei. The rate of brain stem stimulation was limited to 0.5–1 Hz. We noted that at brain stem stimulation rates above 1 Hz, the amplitude of the antidromically evoked compound action potential was enhanced. This would reflect increased excitability and PAD, presumably due to a temporal summation effect induced by preceding brain stem stimuli.

Electrodes were also stereotaxically placed (with histological verification) in the following structures for subsequent conditioning stimulation: PGM, contralateral thalamic nucleus ventralis posteromedialis (VPM), nucleus raphe magnus (NRM) and adjacent reticular formation. Bipolar stimulation of these three sites, plus IO, TP and facial skin, were used as conditioning stimuli (10 ms, 400-Hz train, or 200 ms, 100-Hz train, or single 0.2-ms pulse). Mechanical stimuli applied to the facial skin were

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also used, and included an alligator clamp described as noxious when applied to human skin, a brush and an electronically controlled mechanical probe. The presynaptic modulation by these various conditioning stimuli of TP afferents was determined by measuring a change in their antidromic excitability. An increase in amplitude of the antidromically evoked compound action potential or increase in the probability of antidromic activation of single TP units, were used as measures of PAD[8,16,18].

Table 1 Incidence of PAD produced in TP afferent endings in nucleus oralis or nucleus caudalis by various conditioning stimuli

Conditioning stimulus	Oralis	Caudalis
PGM	66% (47)	64% (64)
NRM	83% (18)	73% (37)
VPM	90% (10)	76% (25)
IO	81% (11)	68% (19)
TP	87% (14)	75% (20)

The numbers in parentheses indicate the number of units tested with each conditioning stimulus.

The following criteria were used to distinguish single-unit activity from compound action potentials: the clear and consistent shape of the former, displaying 'all-or-none' firing at its threshold for antidromic activation, with a constant latency and ability to follow high-frequency (100 Hz) stimulation (to exclude the trigeminal tract reflex[19] which we observed occasionally). A total of 150 units were recorded in TP or the trigeminal ganglion. Antidromically induced compound action potentials and single-unit activity had latencies ranging over 2–6 ms, consistent with TP afferents conducting primarily in the $A\delta$ range[14,20].

All the conditioning stimuli used were capable of inducing PAD of TP afferent endings in oralis or caudalis as judged by changes in the antidromic excitability of compound action potentials and single units recorded either in the trigeminal ganglion or ipsilateral TP. There was no selective presynaptic modulation of the small-diameter (TP) afferents by large afferent fibre activation, and our findings of peripherally induced PAD are supported by an earlier report of PAD in TP afferents[20]; however, centrally evoked PAD of TP afferents has not previously been reported (Table 1). Not every stimulation site was effective for every unit, even in the same animal and using the same conditioning and test sites. In general, the incidence of PAD was somewhat lower for endings in caudalis than for those in oralis (Table 1), in agreement with previous reports[16,19] of PAD in trigeminal afferents. The time course of PAD in a TP unit is shown in Fig. 1. PAD was detected at conditioning-test intervals greater than 20–30 ms, was maximal at around 50 ms and lasted for 100–500 ms.

In 22 TP units showing PAD with PGM, NRM or high-intensity skin conditioning stimulation we also tested the effect of naloxone administration (0.4 mg per kg, intravenously); 14 of these 22 units were antidromically excited from caudalis, and eight from oralis. We chose these 22 units for testing because of their consistent and distinctive shape, and the PGM and NRM conditioning stimuli were set at their lowest intensity for producing a reliable PAD effect. Thus, conditions were made as favourable as possible for detecting the reversal of PAD with the opiate antagonist naloxone that would be expected if the presynaptic excitability change induced by these stimuli is related to the action of endogenous opiate-related peptides. The PAD effect was monitored over a period of 25 min after the initial intravenous administration of the drug. Naloxone did not produce any consistent change in baseline antidromic excitability of these units, and only in one of the 22 units tested was there any evidence of a naloxone-induced reduction in PAD. In this unit, the PAD was produced in its oralis endings (antidromic latency 2.3 ms) by both PGM and NRM conditioning stimuli. However, this reduction of PAD as a result of naloxone administration could be overcome by a small increase (90–140 μA) in conditioning stimulus intensity. The effect of naloxone lasted for 15 min, but recurred when a second dose of naloxone (0.4 mg per kg) was given 28 min after the initial administration. No reduction in PAD was noted, however, after naloxone administration in any of the other 21 units; seven of the units were tested with a second dose of naloxone (0.4–2.0 mg per kg, 15-30 min after initial dose), but again no reversal of PAD was noted.

In conclusion, the various conditioning stimuli used were capable of producing PAD in TP afferents. Stimuli to PGM and NRM, sites implicated in several studies[2,21,22] as sources of descending influences related to narcotic analgesia, were very effective in producing PAD. This would suggest that the PAD produced from these sites is related to narcotic analgesia. The opiate antagonist naloxone, however, failed to reverse PAD produced by PGM or NRM (or high-intensity facial) stimulation in 21 of 22 units tested. This may indicate that opiate receptors and opiate-related peptides are not involved in presynaptic modulation of nociceptive input from primary afferents[1,4–7]. However, there are alternative explanations. Our sample of TP units was probably biased towards the large-diameter TP afferents, and although unlikely, it is nevertheless conceivable that the TP units we tested with naloxone may have been involved in functions other than nociception[15]. In addition, although PGM and NRM constitute likely candidates for sites producing modulation, the actual origin of the hypothetical enkephalin terminals on the putative presynaptic opiate receptors is uncertain[2,21,22]. Thus, it is possible that our conditioning stimuli may not have activated a presynaptic enkephalinergic pathway. It is also possible that opiate receptors mediate presynaptic inhibition by some process other than PAD[8].

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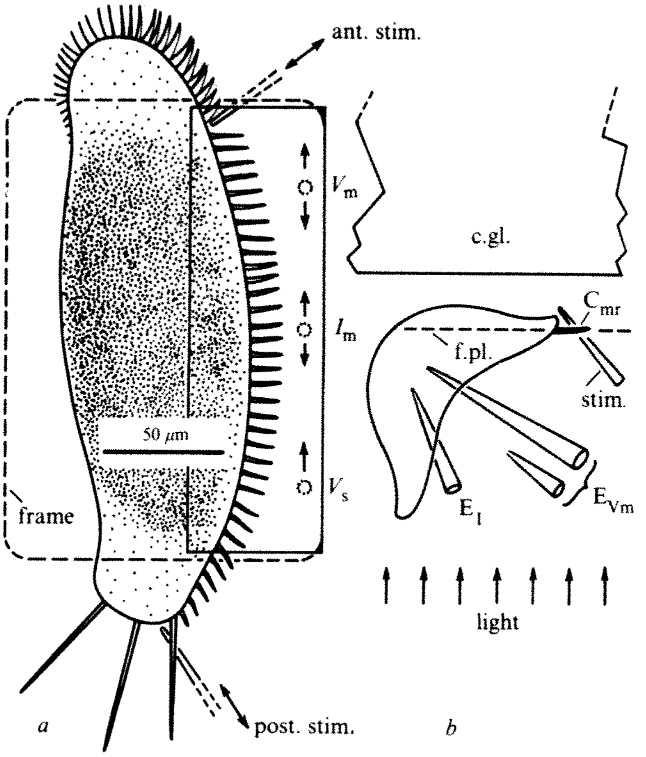
Are receptor-activated ciliary motor responses mediated through voltage or current?

THE locomotion of ciliate protozoa is effected by cilia which modify the direction and frequency of their cyclic beating in response to sensory input from the environment[1,2]. In *Paramecium* and *Stylonychia* mechanical stimulation of the anterior end of the cell induces a Ca^{2+}-dependent depolarising receptor potential which triggers a regenerative Ca^{2+} response[3,4]. Ca^{2+} accumulating in the ciliary space activates the axonemal motor system[5] so that the normal (posterior) direction of the ciliary power stroke is reversed and the frequency is augmented[6]. Posterior mechanical stimulation of the cell elicits a K^+-dependent hyperpolarising receptor potential followed by augmentation of ciliary frequency without reversal[4,7], a mechanism which is less well understood[8]. Because shifts in voltage are associated with loops of electric currents across the membrane, we have examined the crucial signals transmitted from the mechanoreceptive regions to the cilia. We report here that these signals are potentials of either polarity. This is the first report of intracellularly recorded receptor currents.

Fig. 1 *Stylonychia mytilus*, as orientated in the experimental set-up for cinematographic (16 mm, 250 f.p.s.) and electrophysiological recording. *a*, Cell in oblique dorsal view with right marginal cirri (C_{mr}) focused under a compound microscope (Zeiss interference contrast). Vertical oscilloscope deflections of membrane potential (V_m), membrane current (I_m) and driving voltage of piezoelectric stimulator (V_s) optically superimposed on frame. Positions of glass rod for anterior and posterior mechanical stimulation (ant. stim., post. stim.) are indicated. The rectangle marks the outline of photographs in Figs 2–4. Bar, 50 µm. *b*, Position of cell under a cover glass (c.gl.) in the experimental chamber. Electrodes for current passing (E_I) and recording of the membrane voltage (E_{Vm}) were bent to be inserted from below. The position of the glass rod (stim.) was approximately 3 µm from the cell margin before a mechanical stimulus. Experiments were carried out at 18° C.



Single specimens of the freshwater ciliate *Stylonychia mytilus* were mounted on glass microelectrodes (1 M KCl, 40–60 MΩ) under a compound microscope in a solution of 1 mM $CaCl_2$, 1 mM KCl and 1 mM Tris-HCl at *p*H 7.3, to register the stimulus-dependent electrical events as well as the movements of the cirri (Fig. 1). The marginal cirri (10–20 individual cilia, 15–20 µm long) are largely inactivated during the resting state of the cell which occurs while *Stylonychia* 'sits' on substrate surfaces, whereas other compound cilia (membranelles) beat rapidly to produce a feeding water current. Activation of the marginal and ventral cirri produces forward and backward 'walking' locomotion[9]. The experimental cells assumed a steady resting stage 2 min after fixation by two intracellular electrodes (Figs 2–4, leftmost frames) with a resting potential of −51 ± 3 mV.

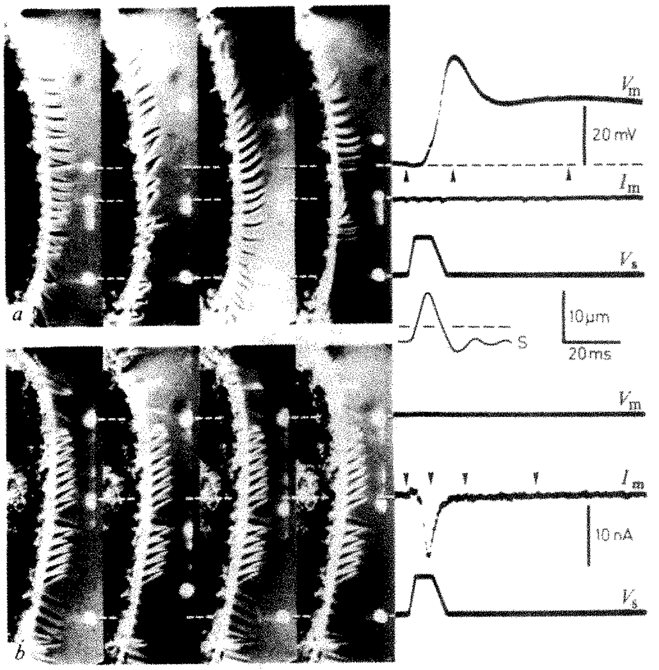


Fig. 2 Electrical and ciliary motor responses to mechanical stimulation of the anterior end of the cell. *a*, Sequence of frames (from left to right) to illustrate the onset of beating of the cirri towards the cell anterior following application of the mechanical stimulus. Oscilloscope traces (right) are graphically joined to signals on photographs (left). Arrowheads mark occurrence of represented frames (4th frame, 140 ms after start of stimulus). Actual tip excursion of needle (S) is indicated below trace of driving voltage (V_s); dashed line limits dead space between resting needle and cell surface[4]. Note activation of cirri during the rising slope of the action potential which rides on top of a receptor depolarisation. Artefacts on current trace (I_m) were picked up from flashes for film exposure. *b*, Absence of motor responses of the cirri after the same mechanical stimulus during voltage clamp of the resting potential. Current trace shows inward receptor current. Conventional oscilloscope display in *b* was recorded directly after filmed sequence to remove amplified flash artefacts from the current trace.

Mechanical stimuli applied to the anterior membrane by the tip of a fine glass rod induced a ciliary motor response within 6–9 ms, coincident with the rising slope of a membrane depolarisation. This electrical response had an early receptor component followed by an action potential[10] (Fig. 2*a*). The activated cirri swung anteriorly to perform several vigorous beating cycles (up to 60 Hz) with the power strokes orientated anteriorly. There was no activation of the cirri when the cell was similarly stimulated while the membrane voltage was clamped to the resting potential by negative feedback to remove the voltage signal (Fig. 2*b*). In this case the anterior mechanical stimulus produced an inward membrane current which rose to a peak (up to 10 nA) within 5 ms and decayed with a time constant of 3 ms, a fraction of the time constant of the decaying depolarising

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receptor potential reported elsewhere[4]. The result suggests that a depolarising receptor potential from a brief conductance increase of the anterior receptor channel passively invades the cilia[11] to activate the voltage-sensitive channel of the ciliary membrane[12,13].

Mechanical stimulation of the cell posterior elicited a ciliary motor response (up to 45 Hz) within 10–13 ms together with a hyperpolarising receptor potential (Fig. 3*a*, 11 ms risetime to 26 mV amplitude, 35 ms time constant of decay). In contrast to the effects of depolarising stimuli, the first (Fig. 3*a*, 2nd frame from left) and subsequent ciliary power strokes were directed towards the posterior end of the cell. On the other hand, on stimulation with the membrane potential clamped to its resting value, the cirri remained unaffected, while a strong outward receptor current (Fig. 3*b*, 10 nA, 5 ms risetime, 7 ms time constant of decay) was recorded. The data shown in Figs 2 and 3 suggest that the ciliary apparatus can specifically respond to receptor-dependent depolarisation or hyperpolarisation, but not to the ionic currents accompanying receptor-site activation.

Motor effects of three types of inward membrane currents of similar amplitudes were examined to test the latter hypothesis.

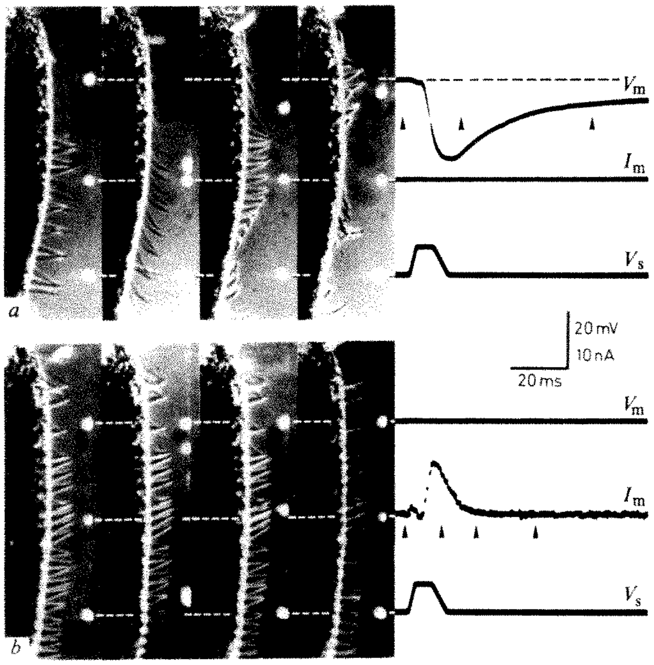

Fig. 3 Effects of posterior mechanical stimulation. *a*, Resting cirri (frame at left) start beating towards the rear of the cell after the occurrence of a hyperpolarising receptor potential (4th frame, 236 ms after stimulus onset). *b*, With the membrane voltage clamped at the resting potential, the same mechanical stimulus gives rise to an outward receptor current (I_m), but the compound cilia remain in the pre-stimulus resting position.

Although an inward receptor current could not activate the cirri in the absence of membrane voltage displacement (Fig. 2*b*), the early inward current due to a depolarising voltage step was accompanied by a reversal response of the cirri (Fig. 4*a*). Inward current from a hyperpolarising voltage step occurred in conjunction with ciliary activation towards the posterior of the cell (Fig. 4*b*). Comparison of Figs 2–4 unequivocally confirms signal transmission to the cilia by means of changes in voltage, not membrane currents. The failure of an inward receptor current to activate the cilia is interesting, as this current is carried by calcium. Estimates of the total ciliary fluid space and the somatic volume (3×10^{-9} cm^3, 4×10^{-7} cm^3; H.M., unpublished), show that a receptor current of 10 nA restricted to the cilia increases the intraciliary Ca^{2+} concentration beyond 10^{-4} M, and raises the internal concentration by 3×10^{-6} M, if Ca^{2+} is evenly distributed in the total cellular space. Both concentrations are sufficient for ciliary reversal[5,14]. Thus, we suggest that Ca^{2+}-receptor channels in *Stylonychia* occur exclusively on non-ciliary membranes. A K^+-dependent hyperpolarising mechanoreceptor channel was also localised on the somatic surface in deciliated *Paramecium*[13], whereas membrane mutants of *P. caudatum* which fail to produce membrane electrogenesis and ciliary reversal[15] exhibit both types of receptor potentials on mechanostimulation[16]. Studies in wild-type *P. caudatum* (A. Ogura and H.M., unpublished) establish inward and outward mechanoreceptor currents and suggest that these currents are absent from the ciliary surfaces.

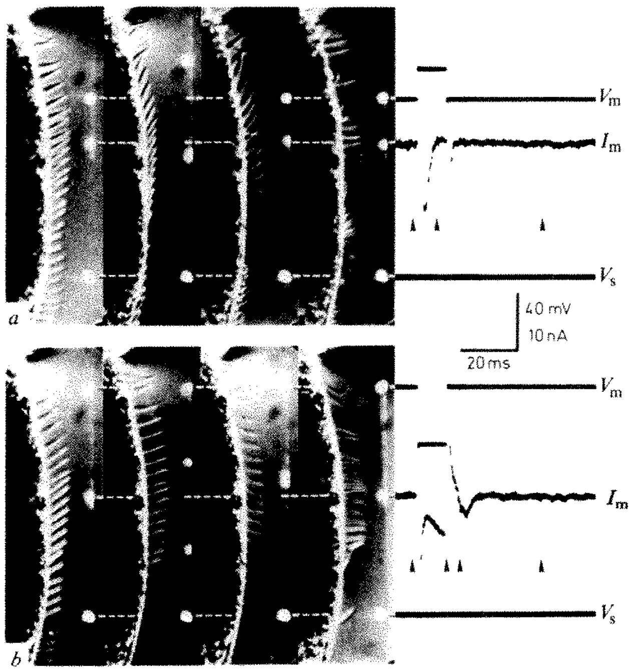

Fig. 4 A test of the adequacy of electrical signal for ciliary activation. *a*, A depolarising voltage step (V_m) produces an early inward current (I_m) and causes the cirri to beat towards the cell anterior (4th frame, 158 ms after voltage stimulus). *b*, A hyperpolarising voltage step accompanied by 'late inward' current activates cyclic beating of the cirri towards the posterior end of the cell. Note that the polarity of the voltage signal determines the ciliary response; the polarity and the size of the membrane current, which is similar to that in Fig. 2*b* (no ciliary activation), is not a signal recognised by the cilia.

We conclude that activation of the ciliary motor response through mechanical stimuli involves the following steps: (1) increase in conductance of the somatic sensory channel; (2) passive spread of the receptor potential into the cilium; (3) conductance modification of the voltage-sensitive Ca^{2+} channel; (4) change in intraciliary Ca^{2+} concentration; (5) Ca^{2+}-dependent activation of the ciliary axoneme.

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Site of 1,25(OH)$_2$ vitamin D$_3$ synthesis in the kidney

FRASER AND KODICEK[1] have reported, and others hae confirmed[2–4], that the kidney is the exclusive site of synthesis of 1,25-dihydroxycholecalciferol (1,25(OH)$_2$D$_3$). The 1-α-hydroxylation of 25-hydroxycholecalciferol (25-OH-D$_3$) occurs in mitochondria[1,4,5] and is influenced by cytosol[6,7], although conflicting data have been reported[8]. Preparations of whole tubules from the cortex possess 1-α-hydroxylase activity[10] but the precise localisation of the enzyme along the nephron is not known. In the dog, no evidence of 1-α-hydroxylase was found in medulla, enzymatic activity being observed only in cortical preparations[5]. In mice given systemic injections of ^{3}H-vitamin D$_3$ autoradiography shows the tracer to be present in the proximal tubules[9]. However, whether hydroxylation occurred at this site was not established. Using single nephron preparations, we have now demonstrated that the major and probably exclusive site of synthesis of 1,25(OH)$_2$D$_3$ is the proximal tubule.

One-day-old white Leghorn cockerels were raised in the dark on a rachitogenic, vitamin D-deficient diet (ICN) containing 1.74% Ca and 1.24% inorganic phosphate (P$_i$). Controls of the same strain were maintained on a normal diet, in daylight. Rachitic chickens were distinguished from controls by their stunted growth, their weakness and the paucity of their feathers. Tubules were prepared by a modified technique of Imbert[11]. After an intravenous injection of heparin, 42 64-day-old animals were bled, and the renal arteries were perfused via the aorta with a cold solution of collagenase (1 mg ml^{-1}) in modified Hank's solution containing 1.0 mM Ca. When completely blanched, the left kidney was removed. Thin sagittal slices were cut, from which minute fragments of tissue (0.5 × 0.5 × 3 mm), involving both superficial and deep parts of the kidney, were isolated and digested in collagenase solution (Type 1 from *Clostridium histolyticum*, Sigma) for 1 h at room temperature. Tubules were dissected at 0 °C, in Hank's modified solution.

The kidney of the chicken consists of three lobes, each divided into numerous lobuli. Each lobulus consists of a cortical part (under the kidney surface) containing nephrons which lack loops of Henle, and a medullary part containing nephrons which have short or long loops of Henle. A superficial nephron consists of a glomerulus, proximal tubule and distal tubule connected to the cortical collecting tubule[12–15]. The early part of the proximal tubule (PCT), is convoluted, thick and relatively friable after digestion with collagenase. The PCT continues with a series of parallel hairpin loops, initially thick (thick loop cortex), and later thin (thin loop cortex), which finally connect to the branched collecting tubule (collecting cortex) through a thin distal convoluted tubule (DCT). The division between the DCT and the collecting cortex was difficult to distinguish so these two segments were dissected and tested together. Medullary nephrons were less readily digested by collagenase, and could be dissected as successfully as those of the cortex. Only small bundles of terminal collecting tubules in medulla were isolated satisfactorily. because of the resemblance of these terminal collecting tubules with the papilla in mammals, these structures were classified as papillary collecting tubules (collecting papilla).

After dissection, fragments of individual tubules were grouped according to their morphological characteristics until a total length of 4–16 mm per group was accumulated. Each group of tubules was kept at 0 °C in a drop of modified Hank's solution containing 0.25 mM of Ca, in the depression of a bacteriology slide. Tubules were photographed to measure their length and incubated in 0.6 to 1.0 µl of incubation medium (IM) for 60 min at 35 °C. The final composition of the incubation medium was: malate 10 mM, Tris-HCl 100 mM, MgCl$_2$ 4 mM, EDTA 0.25 mM, glucose-6-phosphate 5 mM, NADP 0.33 mM, glucose-6-phosphate dehydrogenase 600 units l^{-1}, bovine serum albumin 1%. 25-Hydroxy-(26,(27)-methyl^{3}H)-cholecalciferol (^{3}H-25-OH-D$_3$, Roche), 95–110 Ci mmol^{-1} was added to the preparation (the organic solvent for the labelled compound was evaporated under nitrogen previously). The 25-OH-D$_3$ concentration in the incubation medium varied from 135 to 560 nmol l^{-1}. After incubation, the contents of the slide depression were transferred to chromatography paper impregnated with silica gel (Whatman SG 81) and dried under nitrogen. The slide depressions were rinsed 4 times with 5 µl of chloroform–methanol (1 : 2) and the washings were also transferred to the chromatography paper and dried. The chromatograms were developed in the dark for 16–18 h using chloroform–ethylacetate–benzene (40 : 50 : 10) according to the method of Bikle and Rasmussen[10]. They were then dried in air, cut into 2-cm^2

Table 1 1,25(OH)$_2$D$_3$ synthesis along the nephron

Segment		25 OH D$_3$ in IM (nmol l^{-1})	25 OH D$_3$ recovery (fmol)	1,25(OH)$_2$D$_3$ produced (fmol)	1,25(OH)$_2$D$_3$ (fmol mm^{-1})	1,25(OH)$_2$D$_3$ (fmol µg^{-1} protein)	% Conversion
	PCT (11)	334.6	89.7	0.22	0.03	0.20	—
		± 48.7	± 7.2	± 0.12	± 0.03	± 0.20	—
	Thick loops	347.8	104.8	0.08	−0.01	−0.09	—
	cortex (5)	± 77.7	± 14.0	± 0.03	± 0.00	± 0.06	—
	Thin loops	358.1	108.7	0.00	0.01	0.09	—
Control	cortex (7)	± 63.7	± 10.4	± 0.03	± 0.00	± 0.14	—
	Distal and collecting	323.7	86.8	−0.03	−0.01	−0.13	—
	cortex (9)	± 53.7	± 11.5	± 0.02	± 0.00	± 0.13	—
	Collecting papilla (1)	203	73.2	—	—	—	—
	PCT (19)	299.2	72.8	18.1	1.76	12.3	21.0
		± 29.4	± 1.7	± 2.0	± 0.19	± 1.4	± 2.3
	Thick loops	261.6	78.3	12.8	1.73	12.4	14.3
	cortex (8)	± 33.5	± 3.3	± 3.1	± 0.33	± 2.4	± 2.9
	Thin loops	346.1	121.9	0.69	0.05	0.89	0.79
Rachitic	cortex (7)	± 66.6	± 19.6	± 0.61	± 0.06	± 1.00	± 0.60
	Distal and collecting	356.0	116.4	0.16	0.02	0.28	0.37
	cortex (19)	± 24.7	± 16.4	± 0.10	± 0.01	±0.20	± 0.37
	Collecting papilla (4)	256.0	84.2	0.43			0.01
		± 28.1	± 23.8	± 0.25	—	—	± 0.00

Values are means ± s.e.m. IM, incubation medium. Values in parentheses are numbers of groups of tubules tested.

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pieces, and dispersed directly into scintillation vials containing 15 ml of toluene based scintillation fluid. The amount of vitamin D metabolites was expressed in fmol based on the original specific activity of the ^3H-25-OH-D_3. Approximately 50% of the total radioactivity was recovered.

The chromatographic properties of the vitamin D metabolites were established using labelled authentic compounds: mean mobility of $1,25(OH)_2$-D_3/25-OH-D_3 ratio was 0.50 ± 0.008 (s.e.m.). In six experiments with rachitic chickens, 10 μg each of carrier 25-OH-D_3 and $1,25(OH)_2$-D_3 were cochromatographed with the proximal tubule preparation, after incubation with ^3H-25-OH-D_3. Unlabelled metabolites were localised on the chromatogram either by spraying with a solution of 10% phosphomolybdate in ethanol or by exposure to iodine vapour; the radioactive metabolites were then localised as previously described. The chromatographic properties of the authentic carrier sterols and the labelled metabolites formed by the tubules were identical (Fig. 1).

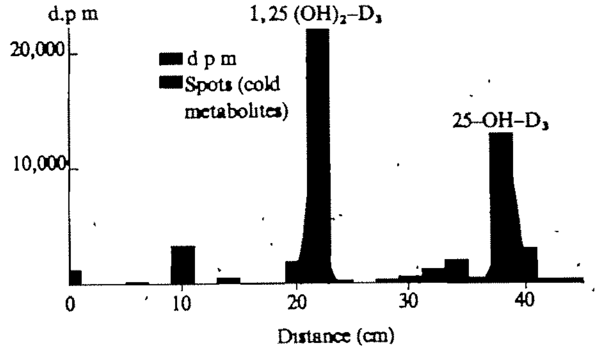


Fig. 1. Cochromatogram of a PCT preparation from a rachitic chicken after incubation with ^3H-25-OH-D_3, and a mixture of the two unlabelled vitamin D_3 metabolites. 25-OH-D_3 and $1,25(OH)_2$-D_3. The labelled metabolite produced by the tubular preparation exhibits identical migration characteristics to $1,25(OH)_2D_3$.

The mean protein content of the tubules was determined in three 10-week-old normal chickens, three 4-week-old normal chickens, and three 7-week-old rachitic chickens. Protein content was measured by a microtechnique described by Butcher and Lowry[16]. Fragments of tubules were processed and photographed as described above. As the protein contents of proximal convoluted tubules and thick loops of the cortex were similar, the results were pooled. Similarly, thin loops of the cortex, distal convoluted tubule, and cortical collecting tubules were combined and assayed together. Protein digestion in tubular preparations and in standards of bovine serum albumin was performed in 1 μl 6 M HCl, under oil, at 120 °C for 4 h. Fluorescence was developed by mixing the digested proteins with 100 μl of a mixture of 1 mM orthophthalaldehyde and 2 mM β-mercaptoethanol in 100 mM sodium tetraborate buffer at *p*H 10. The degree of fluorescence was measured after dilution in 500 μl of 0.5 M NaOH, using an Aminco-Bowman microfluorometer.

Plasma Ca and P_i were 3.33 ± 0.19 mEq l^{-1} and 1.55 ± 0.24 mmol l^{-1} respectively in rachitic chickens, compared to 4.07 ± 1.03 mEq l^{-1} and 2.61 ± 0.30 mmol l^{-1} in controls. The protein content of proximal (PCT thick loops cortex) and distal (thin loops cortex, collecting cortex) segments of the nephron were (μg mm^{-1}) 0.23 ± 0.4 and 0.11 ± 0.00 in 10-week-old chickens, 0.13 ± 0.00 and 0.06 ± 0.005 in 4-week-old chickens, 0.14 ± 0.01 and 0.06 ± 0.01 in rachitic chickens who were 5- to 9-weeks-old. Because of their size, 4-week-old chickens were used as controls, and the values of $1,25(OH)_2$-D_3 produced per mm tubule were converted, in the two series of animals, to fmol per μg of protein, utilising the corresponding tubular protein content.

The levels of 1-α-hydroxylase activity within the nephron segments are given in Table 1 and Fig. 2. The only significant production of $1,25(OH)_2$-D_3 occurred in the PCT and the thick loops of the cortex, in rachitic chickens: 18.1 ± 2.0 fmol and 12.8 ± 3.1 fmol respectively, that is, 1.76 ± 0.19 and 1.73 ± 0.33 fmol per mm of tubular length, or 12.3 ± 1.3 and 12.4 ± 2.4 fmol per μg-protein. No $1,25(OH)_2$-D_3 was produced in the remaining nephron segments. The degree of 25-OH-D_3 transformation to $1,25(OH)_2$-D_3 varied between animals. The mean percentage transformation was 21.0 ± 2.3% and 14.3 ± 2.9% in PCT and the thick loops of cortex, with a maximum of conversion of 53%.

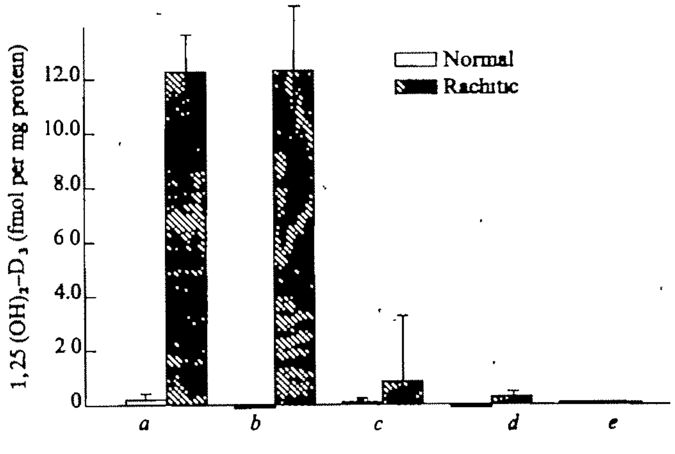
These studies demonstrate that, in vitamin D-deficient chickens, renal 1-α-hydroxylase activity is found exclusively in the proximal tubule. The absence of enzyme activity in the rest of the nephron may be assumed, unless other active segments are too short to produce detectable amounts of enzyme activity with this technique. If a distal site of enzyme activity exists, its activity is negligible in comparison with that found in the proximal tubule.

Our results agree with those reported by Midgett *et al.*[5] who observed 1-α-hydroxylase activity in the cortex of the canine kidney but none in the medulla. In addition, the present data localises the site of the enzyme activity in the cortex, providing evidence that the enzyme is located exclusively in the proximal tubule.

Using homogenates of chicken kidney cortex, Midgett[5] has reported a production of 10 pmol of $1,25(OH)_2$-D_3 per mg of protein after 2 h of incubation. Henry *et al.*[17] using kidney homogenates has reported a production of approximately 1 pmol of $1,25(OH)_2$-D_3 per min per mg of protein. In the present study the proximal tubule exhibited a wide range of enzyme activity depending on age and on various other factors. The mean production of $1,25(OH)_2$-D_3 was 12.3 fmol per μg of protein during a 60-min incubation, a value which is of the same order of magnitude as those found in other laboratories.

The proximal tubule is responsible for most of the P_i reabsorption. In this segment, PTH[18], and in some species, calcitonin[19], stimulate cyclic AMP synthesis which interferes with P_i transport. PTH and cyclic AMP enhance the synthesis of $1,25(OH)_2$-D_3 (refs 20–23) and, conversely, vitamin D and its metabolites increase P_i reabsorption[24–26], probably by interfering with adenyl cyclase activity[27]. The localisation of 1-α-hydroxylase activity within the proximal tubule where the above factors converge their influence, would suggest that $1,25(OH)_2$-D_3 may participate in the regulation of tubular P_i transport.

Fig. 2 $1,25(OH)_2D_3$ synthesised by various segments of the nephron of normal and rachitic chickens. Columns indicate the mean values and bars the standard errors. A significant amount of the vitamin D_3 metabolite was found only in PCT and thick loops of the rachitic chickens. The results are expressed in fmol of $1,25(OH)_2D_3$ per μg of tubular protein. *a*, PCT; *b*, thick loop cortex; *c*, thin loop cortex; *d*, DCT; *e*, collecting papilla.



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Rapid transbilayer diffusion of 1,2-diacylglycerol and its relevance to control of membrane curvature

SHEETZ AND SINGER have proposed that the actions of certain amphipathic drugs in producing curvature of the membrane of human red blood cells (RBCs) could be explained by assuming that these drugs selectively entered one of the leaflets of the lipid bilayer, thus causing lateral expansion of that leaflet relative to the other[1]. The membrane was therefore forced to curve in order to accommodate this extra material and the direction of this curvature was predictable, leading to production of either stomatocytes or echinocytes. Membrane curvature, leading to the formation of stomatocytes or echinocytes, can also be produced by subjecting human RBCs to phospholipase C attack[2–5]. In this report we attempt to explain these phospholipase C-induced changes in terms of Sheetz and Singer's model, taking into account the observation that 1,2-diacylglycerol (the product of phospholipase C action), unlike phospholipids, can migrate rapidly across the membrane bilayer. Rapid transbilayer migration of 1, 2-diacylglycerol has previously been inferred from the observed increase in the rate of synthesis of phosphatidate in intact RBCs that have been exposed to phospholipase C: this phosphatidate is formed from diacylglycerol and cytosolic ATP by diacylglycerol kinase, presumably at the inner surface of the membrane[2]. We have now used this procedure, in which the endogenous diacylglycerol kinase of the erythrocyte is used to trap 1, 2-diacylglycerol appearing on the inner surface of the red cell membrane, to compare the time courses of phospholipase C-catalysed production of diacylglycerol and of the appearance of phosphatidate, and thus obtain an estimate of the rate of transbilayer migration of 1, 2-diacylglycerol.

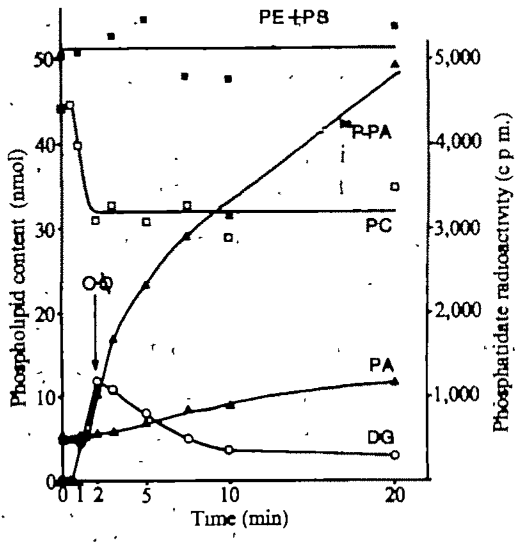


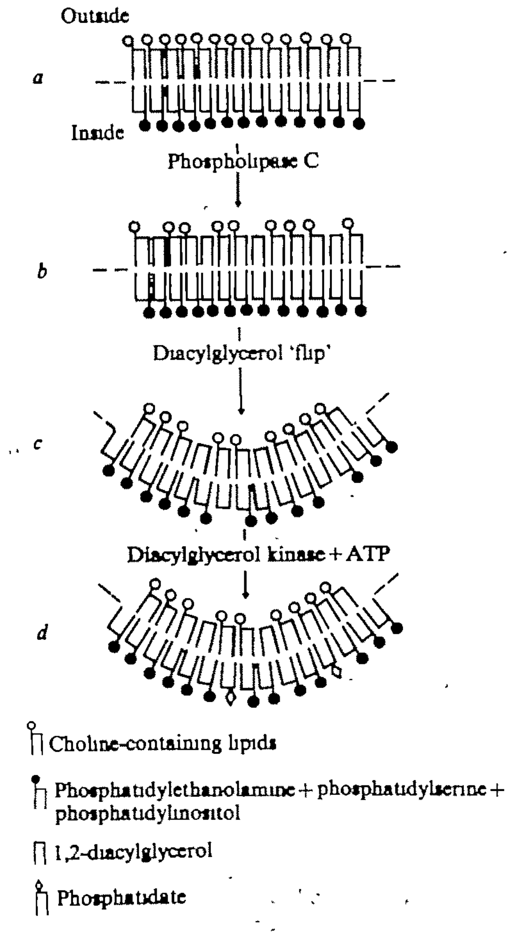
Fig. 1 Time course of production of 1,2-diacylglycerol and phosphatidate in human RBCs exposed to phospholipase C. Outdated human RBCs were washed and prelabelled for 90 min at 37 °C with ^{32}P-Pi (10 μCi ml^{-1}) at a cell concentration of 2×10^9 ml^{-1} in a HEPES-buffered Ringer solution as described previously[2]. Phospholipase C (*C. Welchii* freeze-dried culture filtrate type A, batch AD1051A, Wellcome, Beckenham, UK) was added at a final concentration of 10 μg ml^{-1} and was inactivated after 2 min incubation by addition of *o*-phenanthroline to 1 mM final concentration; 0.5-ml samples of the incubation were taken at various times and immediately added to 1.9 ml of methanol: chloroform: concentrated HCl 200:100 1. Lipids were extracted as described previously and after concentration were separated according to the procedures of Skipski *et al.*[12] (phospholipids) and Freeman and West[13] (neutral lipids). Phospholipids were quantified by the procedure of Bartlett[14] following digestion with perchloric acid, and ^{32}P radioactivity in phosphatidate was measured by Čerenkov counting in a Searle liquid scintillation counter. The 1,2-diacylglycerol spots from the chromatogram were excised and heated at 100 °C for 1 h with 1 ml of methanol: concentrated HCl (10:1) under nitrogen in a screw-top tube. After cooling, the samples were dried at 40 °C under reduced pressure. The residue, which contained free glycerol, was silylated under N_2 at 100 °C for 10 min with a mixture of 20 μl pyridine and 20 μl *bis* (trimethylsilyl)-acetamide (Supelco, Bellefonte, Pennsylvania), 5 μl of the resulting mixture was applied to a 1 5-m GLC column (3% OV1 on 80–100 mesh Supelcoport) at an N_2 flow rate of 40 ml min^{-1} and a temperature of 120 °C in a Pye 204 chromatograph. The silylated derivative of glycerol gave a peak at 3.8 min and was quantified by measurement of peak area compared with peaks given by various amounts of standard dipalmitin (Sigma) which had been treated in an identical fashion. Cell lysis was measured in samples of cells that were taken at various times and diluted in an isotonic saline solution containing 1% formaldehyde (to fix the cells) and 1 mM *o*-phenanthroline which inhibits the phospholipase). The cells were spun down and the optical density at 410 μm of a suitable dilution of the supernate was measured. The experiment shown was one of five which gave closely similar results. ■, Phosphatidylethanolamine + phosphatidylserine, □, phosphatidylcholine; ○, 1,2-diacylglycerol; △, phosphatidate; ▲, phosphatidate radioactivity.

Washed human RBCs were incubated with ^{32}P-phosphate to prelabel their ATP pool. They were exposed briefly to phospholipase C and the enzyme was then inactivated with *o*-phenanthroline (see Fig. 1). Samples were taken at various times and determinations made of 1,2-diacylglycerol, phosphatidate and phosphatidylcholine content. Radioactivity in phosphatidate was also measured.

As shown in Fig. 1, there was a lag of about 30 s before the phospholipase began to attack the RBC lipids rapidly. Breakdown of phosphatidylcholine and accumulation of 1,2-diacylglycerol then continued until the enzyme was inactivated with *o*-phenanthroline at 2 min. The maximal rate of phosphatidate synthesis was about 1.5–2.0 nmol min^{-1} 10^{-9} cells, and this corresponded to the maximum rate observed in other experiments when diacylglycerol kinase was assayed in lysed cells (unpublished) or in membranes[6]. In the experiment in Fig. 1, cell lysis was $<5\%$ in phospholipase-treated cells after 20 min, so that the great bulk of the 1,2-diacylglycerol must have initially been derived from outer-leaflet phosphatidylcholine and must then have traversed the membrane to gain access to diacylglycerol kinase and ATP. This assumption is supported by fatty acid determinations (unpublished) which showed that the compositions of 1,2-diacylglycerol and of phosphatidate derived from it, were not distinguishable from the composition of phosphatidylcholine. The maximum rate of synthesis of phosphatidate was observed about 1 min after achievement of the maximal rate of production of 1,2-diacylglycerol, suggesting that 1,2-diacylglycerol diffused into the inner leaflet of the cell membrane within about 1 min of its production in the outer leaflet. This rate is very much faster than the rates of transbilayer diffusion for erythrocyte phosphatidylcholine, the half-time of which for 'flip-flop' has been estimated as between 0.5 and 6.5 h (refs 7–9).

A schematic diagram to illustrate the possible morphological consequences of this fast diffusion of diacylglycerol is shown in Fig. 2. Formation of diacylglycerol from phospholipid in the outer leaflet (Fig. 2*b*) is followed by rapid movement of diacylglycerol into the inner leaflet (Fig. 2*c*), thus causing a simultaneous contraction of the outer leaflet and expansion of the inner leaflet and a resulting tendency for the membrane to curve inwards. Phosphorylation of the internal 1,2-diacylglycerol (Fig. 2*d*) may further expand the inner leaflet as the utilisation of the diacylglycerol of the inner leaflet would favour the continued diffusion of diacylglycerol in from the outer leaflet. Also as phosphatidate would probably occupy a larger area than 1,2-diacylglycerol. However, the phosphorylation of diacylglycerol should not be the essential step in the induction of membrane curvature; it should be sufficient that phospholipid is broken down on one side of the membrane to give an accumulation of a rapidly migrating species such as diacylglycerol. This model therefore provides a rationale for the membrane invagination which follows attack on RBCs by externally added phospholipase C (ref. 2) and also for the evagination of membrane which follows activation by Ca^{2+} of the endogenous phospholipase C that attacks the polyphosphoinositide of the inner leaflet to give an accumulation of 1,2-diacylglycerol[3–5].

Fig. 2 Membrane bending induced by conversion of phospholipid to 1,2-diacylglycerol in one leaflet of a lipid bilayer, followed by diffusion of diacylglycerol into the other leaflet. *a*, Untreated bilayer; *b*, bilayer treated with phospholipase C on the outside; *c*, equilibration of diacylglycerol across the bilayer with consequent induction of bilayer curvature; *d*, phosphorylation of diacylglycerol to form phosphatidate on the inner face of the bilayer.


Outside
a
Inside
Phospholipase C
b
Diacylglycerol 'flip'
c
Diacylglycerol kinase + ATP
d
Choline-containing lipids
Phosphatidylethanolamine + phosphatidylserine + phosphatidylinositol
1,2-diacylglycerol
Phosphatidate

In both of these situations, the membrane curvature induced by asymmetric modification of the lipid bilayer brings membrane surfaces closer together and thus is an essential step which precedes the membrane fusion events which cause the release, either internally or externally, of membrane vesicles. As has been suggested before[4], diacylglycerol-rich areas of membrane may promote the membrane fusion process, perhaps by formation of hydrophobic regions segregated from the bilayer[10].

Although this model for the induction of membrane curvature by the unilateral conversion of phospholipids to diacylglycerol applies to the erythrocytes of man and some other species[2–5], similar principles might govern the control of membrane curvature and fusion in other physiological systems. The phospholipases C which have been identified in animal cells are activated by Ca^{2+} and act specifically on phosphoinositides[3,11], suggesting that inositol lipids, and the phospholipases C that attack them, may have an important role in the control of membrane curvature and fusion *in vivo*, especially in processes (for example, exocytotic secretion) that are brought about by an increase in the intracellular Ca^{2+} ion concentration.

We should also consider the possibility that conversion of phospholipids to other species which can rapidly cross a membrane bilayer (for example, fatty acids) may also affect membrane curvature in a similar fashion.

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Whitepox virus isolated from hamsters inoculated with monkeypox virus

SINCE the eradication of smallpox in Equatorial Africa several 'new' pox viruses have been isolated in our laboratory from materials collected by WHO field workers—monkeypox virus from an affected individual and the 'whitepox' viruses from apparently healthy monkeys and rodents Monkeypox virus is not widespread but occasionally infects man and has caused death: 33 such cases have occurred in Africa, with 6 deaths. It is not known whether whitepox viruses are pathogenic for man but by laboratory markers they are indistinguishable from variola virus[1]. We report here that a whitepox virus identical to the whitepox virus found in feral monkeys and rodents in Equatorial Africa has now been isolated from the kidney and lungs of golden hamsters with an asymptomatic form of infection caused by intracordial inoculation with monkeypox virus. This result supports the hypothesis that natural whitepox viruses are white clones always present in populations of monkeypox virus.

Isolates from monkeypox virus population clones identical with whitepox viruses were shown to be stable and did not change their properties with serial passages on the chorioallantoic membrane of developing chick embryos[2]. As cloning is an artificial laboratory procedure we attempted to determine whether this change in monkeypox virus population occurs in a living host. We suspected the existence of such a mechanism on the basis of whitepox virus detection in African rodents (*Mastomys natalensis*) insusceptible to this virus.

Three-week-old golden hamsters (*Mesocricetus auratus*) inoculated intracordially with 0.5 ml of chorioallantoic monkeypox virus culture (Copenhagen strain) at a dose of 10^6 pock-forming units (PFU) were used as an experimental model. For virus isolation from visceral organs groups of inoculated animals were killed 3 days post infection and at 7-day intervals. At each stage the blood and organs of 10 to 40 animals were tested. Suspensions of 1 part tissue: 2 parts McIlvain buffer solution were prepared from the liver, kidney, lung, spleen, lymph nodes, brain and testis. Viruses were isolated on the chorioallantoic membrane (CAM) of 12-day-old chick embryos; incubation was for 72 h at 35°C. Pox antibodies in the serum were determined in haemagglutination inhibition (HAI) test (with 2 haemagglutination units of vaccinia virus), and in the neutralisation tests on chick embryos by pock reduction on the CAM using standard methods.

Hamsters inoculated with the virus intracordially as well as by intranasal and percutaneous routes showed no clinical symptoms of the infection. Pox antibodies were detected in nearly 50% of the infected hamsters. In animals in which antibodies were present they developed during the second week, reached the maximum by 4–5 weeks and disappeared 8–9 weeks post inoculation. Pox antibody titres were not high (the highest antihaemagglutinin titre did not exceed 80).

Within the first 3 days after inoculation virus was found in the blood and organs of almost all the animals examined. A week later virus was isolated from only 30% of the animals. The number of virus carriers continued to decrease: after 2 weeks 25% carried the virus, after 4 weeks 13%, and after 6 weeks, 9%. At 7 and 9 weeks post infection no virus was isolated from the hamsters. It should be emphasised that two and more weeks post inoculation the virus could be detected only in lungs and/or kidneys of the hamsters and was absent from blood.

Investigation of isolates obtained from the hamsters' organs 3 days, 1 week and later post inoculation showed that in a majority of cases they were identical with monkeypox virus used for inoculation. However, 4 of 35 isolates differed markedly from the parent virus: three had been isolated from kidneys 2, 3½, and 4 weeks post inoculation and one from lungs of the hamsters 4 weeks post inoculation. A more detailed study showed these viruses to be fully identical with natural whitepox viruses isolated from African animals as well as with variola virus. A comparison of two of these isolates with the parental strain of monkeypox virus and whitepox virus Chimp-9 is shown in Table 1.

Further passaging of 'white' isolates on the CAM of chick embryos did not change their properties.

Our results show that 'white' clones can be produced from a monkeypox virus population by a 'natural' mechanism as well as by cloning. In asymptomatic infections of hamsters induced by

Table 1 Comparison of 'white' isolates derived from kidneys of hamsters infected intracordially with monkeypox virus

		Isolates		Reference virus strains	
Markers		$\mathrm{MpW}_{\mathrm{Ham-1}}$	$\mathrm{MpW}_{\mathrm{Ham-2}}$	Whitepox (Chimp − 9)	Monkeypox (Copenhagen)
	Pock morphology†	White, dense	White, dense	White, dense	2 types flat with haemorrhages and single white
Chick embryos*	Lethality LD_{50} (PFU per 0.1 ml)	2.3	2.5	2.2	<1
	Accumulation in liver† (PFU per g tissue)	8.5	8.3	8.7	9.1
	Pock formation at 39°C	None	None	None	Yes
PEK marker		Positive	Positive	Positive	Negative
Neuropathogenicity for white mice§ LD_{50} (PFU per 0.03 ml)		6.3	4.6	4.4	2.7
Rabbit skin test at inoculation (dose, 10^6 PFU per 0.1 ml)	Intradermally	Scarce soft infiltrate	Scarce infiltrate	Scarce infiltrate	Dense infiltrate with necrosis
	On scarified skin	No reaction	No reaction	No reaction	Papulopustular eruption

Pock morphology was determined on CAM.

* Age of chick embryos at inoculation—12 days.

† Incubation post inoculation—72 h at 35°C.

‡ PEK marker—presence of CPE and haemadsorption of chick erythrocytes of the continuous pig embryo cell line infected with virus dose of 10^4 PFU.

§ Neuropathogenicity was determined on 10-day-old white mice at intracerebral inoculation.

monkeypox virus the population of monkeypox virus that persists in the organism undergoes certain changes. It seems likely that 'white' clones initially present at a low level (<5%) are preferentially selected for. This selection process occurs in an unpredictable manner and the factors controlling it are not known. It seems probable that the whitepox viruses detected in feral animals (including those not susceptible to them) are produced through a similar mechanism.

Finally, as whitepox viruses are indistinguishable from variola virus in all laboratory marker tests our results may be considered as a first experimental indication of a possible origin of variola virus from monkeypox virus.

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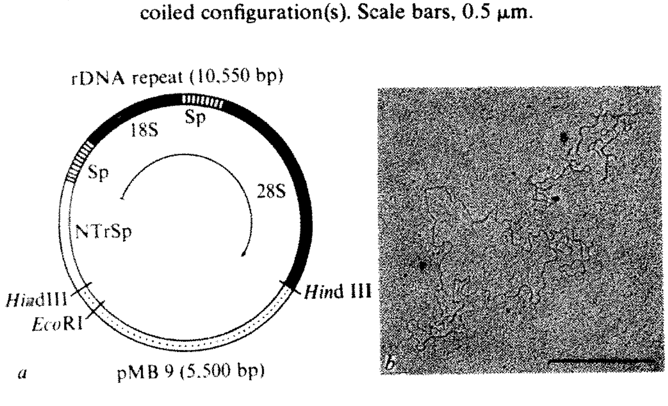
Transcription of cloned *Xenopus* ribosomal genes visualised after injection into oocyte nuclei

DNA CLONING and the direct visualisation of chromatin transcription by electron microscopy provide useful details of the structure and function of genes, but the value of these techniques would be even greater if they could be applied to the same genes or segments of DNA. Our previous work[1] on amplified ribosomal DNA (rDNA) of the water beetle *Dytiscus* showed that rDNA injected into oocytes is correctly transcribed. Gradients of growing rRNA fibrils could be unambiguously recognised as correct transcription when compared to the characteristic transcription pattern observed in the original cell type. We have now extended the usefulness of this technique to recombinant DNA, choosing as an example of a cloned vertebrate gene a single rDNA repeat of *Xenopus laevis* contained in plasmid DNA. The results show that this gene may be transcribed with remarkable fidelity in injected oocytes, and that untranscribed genes are converted into a characteristic chromatin structure.

Fig. 1 *a*, Diagram of pXlr 101. This is a single rDNA repeat of *Xenopus laevis* inserted into the plasmid pMB9 at the *Hin*dIII site. The rDNA repeat (10,550 base pairs) is orientated with the non-transcribed spacer (NTrSp, 2,350 base pairs) next to the *Eco*RI and *Hin*dIII sites of the plasmid. The rDNA coding region is limited at its 3′ end by a *Hin*dIII cut 50 base pairs before the end of the normal 28S transcription region. Normal rDNA transcription initiates and terminates where indicated by the arrow inside the diagram. DNA has been characterised as described in ref. 4. *b*, Electron micrograph of circular recombinant DNA used for injection. DNA was spread out for electron microscopy as described before[27]. The relaxed molecule (r) shown has a contour length of 5.06 μm (see Fig. 2*a*). Most molecules occur in a supercoiled configuration(s). Scale bars, 0.5 μm.



A pMB9-based recombinant plasmid (pXlr 101) was used; its composition[2–4] is summarised in Fig 1*a*. To determine the size distribution of DNA used for injection, purified DNA was spread for electron microscopy (Fig. 1*b*). More than 90% of the molecules were in supercoiled circular configuration. Relaxed circles revealed a homogeneous distribution of contour lengths (mean value 5.19 μm, Fig. 2*a*). To prepare nuclear spreads, oocytes from four *Xenopus* females were each injected with 50 nl DNA solution and a trace of ^{125}I–BSA, aiming for the nucleus[5]. Nuclei were isolated 8–18 h later, counted to identify successful nuclear injections, and spreads for electron microscopy prepared as before[1]. Injected molecules were clearly identified in nuclear spread preparations by the small size of the injected circles[1]. Almost all injected material was found as newly assembled chromatin[6,7], no large masses of 'naked' DNA being observed. Most of the beaded chromatin circles were seen as densely aggregated masses and could be individually identified only in the more dispersed peripheral parts of the preparations. Only clearly detached, open circular, molecules were measured and analysed further.

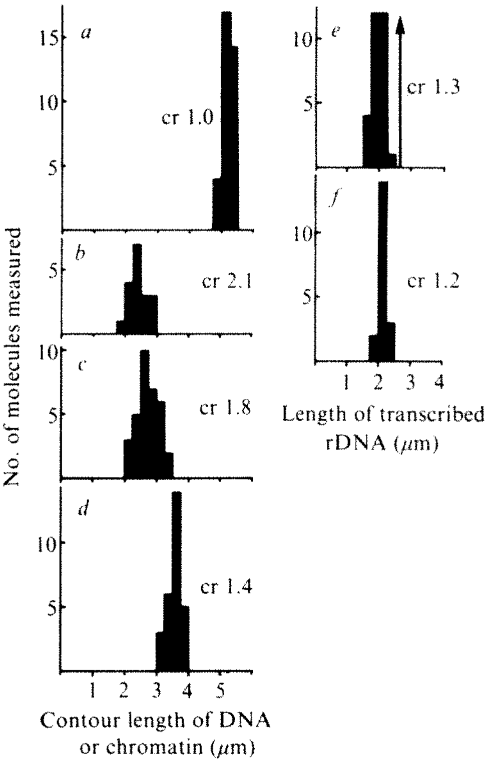


Fig. 2 Length distributions of DNA used for injection and of chromatin recovered from reisolated nuclei. *a*, DNA spread with cytochrome *c*. *b*, Beaded chromatin circles without apparent transcriptional complexes. *c*, Chromatin circles with sparse irregular transcriptional activity. *d*, Chromatin circles with fully transcribed regions, presumably the rDNA insert. The numbers designated cr in each histogram indicate the compaction ratio of DNA in the chromatin circles compared to the length of fully extended pure DNA (*a*). *e* And *f* show the lengths of the fully transcribed chromatin regions which carry transcripts. *e*, Chromatin circles from injected nuclei. *f*, Endogenous amplified rDNA. The arrow in (*e*) indicates the fully extended length of rDNA which can carry transcribed sequences. All measurements shown in *b*–*f* were made on the same single nuclear spread preparation.

Our reasons for claiming that cloned rDNA can be treated normally in injected oocytes depend on its structure as tran-

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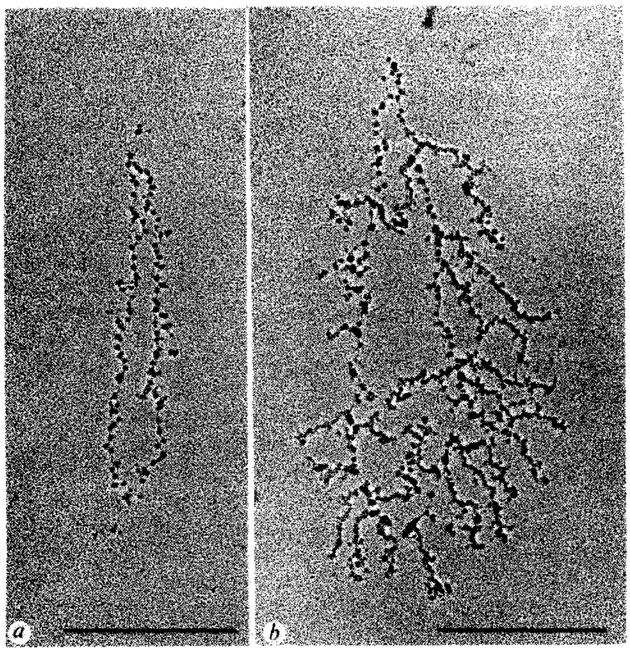

Fig. 3. Spread preparations of injected oocyte nuclei, showing circular chromatin. Oocyte nuclei were injected with purified DNA, incubated for 8 or 18 h (see text); individual nuclear contents were dispersed in low salt medium, and processed for electron microscopy with positive staining and rotary shadowing as before[1]. *a*, A regularly beaded chromatin circle with no apparent transcriptional activity (contour length 2.43 μm). *b*, A typical example of the sparsely transcribed circle (contour length 3.10 μm). Note the irregular spacing of polymerases and the varying length of the lateral fibrils. These circles are taken from the same nuclear preparation as was used for histograms *b* and *c* of Fig. 2.

scribed or untranscribed chromatin. Injected DNA circles can be classified as non-transcribed or transcribed depending on whether lateral RNP fibrils are absent or present respectively.

The great majority of chromatin circles have a regularly beaded ultrastructure (Fig. 3*a*; 80–90 beads per circle; diameter of the particles after positive staining and rotary shadowing, 12–20 nm) and a mean contour length of 2.45 μm (Fig. 2*b*). This suggests that the injected DNA is assembled into a regular nucleosome structure since it appears to have 180–200 base pairs DNA per nucleosome repeat (see ref. 8). There is a 2.1-fold compaction of the original DNA into chromatin as visualised after dispersion in low-salt medium. A similar compaction was obtained after injection of various other samples of circular DNA, including pMB9 DNA and a recombinant pMB9 plasmid containing four *Xenopus laevis* 5S DNA repeats (data not shown). These results are in agreement with calculations made for spread linear chromatin from various eukaryotic cell types[9–11].

The identification of transcriptionally active chromatin is based on the presence of nascent ribonucleoprotein (RNP) fibrils (see ref. 12 for limits of detection and refs 13, 14 for distinction between nucleosomes and RNA polymerases in nuclear spread preparations). Transcriptional complexes on injected circles were observed in 6 out of 21 preparations of spread nuclear contents. On each of these six grids more than 90% of the injected material was assembled into transcriptionally inactive chromatin. Transcriptionally active chromatin was classified as sparsely or densely transcribed according to the packing and arrangement of polymerases and the lengths of the attached RNP fibrils. We have seen about equal numbers of sparsely or densely transcribed chromatin circles. Sparsely transcribed chromatin (see Fig. 3*b* for a typical example) has a regularly beaded structure interspersed with a low number of irregularly spaced polymerases (3–10 polymerases per μm chromatin). The attached RNP fibrils show no graded length distribution and are up to 0.80 μm long. In some cases such circles contain short segments (0.2–1.2 μm) with closely packed polymerases (30–50 polymerases per μm) carrying transcripts of irregular length. The average contour length of these sparsely transcribed circles is 2.77 μm (Fig. 2*c*); this is greater than the mean length of the non-transcribed molecules.

Densely transcribed chromatin has at least 1.6 μm of closely packed polymerases bearing transcripts of gradually increasing length. We interpret the molecules shown in Fig. 4*a*, *b* as examples of correct transcription of cloned *Xenopus* rDNA. Molecules of this kind, of which we have measured 28 examples, show a detailed morphological similarity to the transcription units of endogenous amplified rDNA from the same nucleus (Fig. 4*c*). Circles of the kind shown in Fig. 4*a*, *b* are characterised by having three regions: (1) A densely transcribed region, which includes the 18S, 28S sequences. (2) A non-

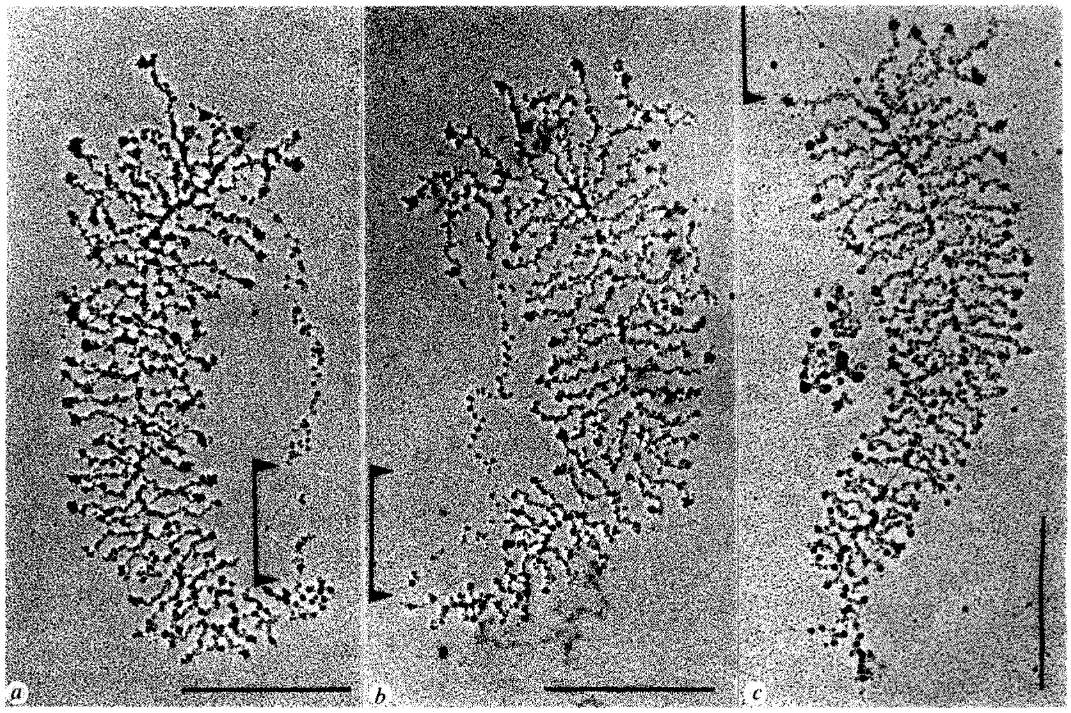

Fig. 4 Ribosomal DNA transcription patterns of injected recombinant plasmid circles (*a*, *b*) and an endogenous rDNA segment (*c*) seen in the same preparation. The morphology of the transcription units is identical by the criteria of density and size of rDNA-attached polymerases, the lengths and gradient arrangement of lateral fibrils, and their terminal coiling which results in dense, knob-like structures. Note the predominantly unbeaded structure of the 'spacer' segments in both figures (arrows).

transcribed stretch of DNA with a 'smooth' ultrastructure: this precedes the densely transcribed 18+28S region, and probably corresponds to the 'non-transcribed' spacer sequences (3) A regularly beaded region without detectable transcriptional activity, presumably representing the pMB9 plasmid DNA. Within the transcribed regions, polymerases are densely packed (30–50 polymerases per μm), and lateral precursor rRNP fibrils are arranged in a typical length gradient, with terminal fibrils up to 0.40 μm in length frequently ending in knob-like structures. The length of chromatin which bears transcripts is 1.63–2.34 μm (mean 1.98 μm; see Fig. 2*e*); this is conspicuously similar to the length of transcribed regions of endogenous amplified rDNA seen on the same preparations (range 1.90–2.39 μm, mean 2.14 μm; see Fig. 2*f*) and reported in other studies[2,15–19]. The 'spacer' segments of injected rDNA are seen as predominantly 'smooth' stretches (mean length 0.63 μm); they are located before the start region (5′ end) of the transcribed regions, and are characterised by a greatly reduced number or complete absence of associated granular particles, in contrast to the beaded structure of the plasmid chromatin adjacent to the 3′ end of the rDNA insertion.

Figure 2*d* shows that the circles containing densely transcribed rDNA (mean contour length 3.61 μm) are compacted 1.4 times compared to the length of the fully extended DNA before injection (Fig. 2*a*). This is considerably less compaction than is seen in non-transcribed or sparsely transcribed rings obtained from the same preparation (Fig. 2*b*, *c*). Circles with a densely transcribed rDNA insert allow a precise comparison of the structural configuration of DNA in transcriptionally active and inactive regions. The 8,200 base pairs of transcribed rDNA is 2.64 μm as pure DNA and 1.98 μm as transcribed chromatin; this represents compaction of 1.3, and shows that transcribed rDNA is almost fully extended (Fig. 2*e*, *f*). A similarly extended DNA configuration can be calculated for most of the smooth 'spacer' regions (see also refs 13, 14, 18, 20).

The precision with which injected rDNA may be transcribed is remarkable The existence of a gradient of transcript lengths suggests that all initiations have taken place at the same point. The typical length of chromatin densely packed with polymerases indicates that transcription is terminating at the correct distance after initiation, as is also implied by the typical length of the longest transcripts. The nucleosome-free region of chromatin immediately in front of the initiation site is characteristic of rDNA transcription. We conclude that a single ribosomal gene repeat contains sufficient information in its DNA sequence to promote accurate transcription, and that this can take place on a relatively short length of DNA, as previously demonstrated biochemically for 5S genes[21] and 4S genes[22,23]. The transcription of the injected rDNA is resistant to low levels of α-amanitin, indicating that it is not transcribed by polymerase II; the accurate morphology of the densely transcribed rDNA suggests that it involves the use of polymerase I.

One of the most interesting general conclusions to emerge from the electron microscopy of nuclear spreads is that the transcription of ribosomal genes is not regulated simply by the supply of RNA polymerase, but seems to be governed by some component which makes a gene either wholly unavailable (closed) or maximally available (open) for polymerase attachment and transcription. Evidence for the existence of such a component has been implicated in previous nuclear spread studies. In amphibian oogenesis[24], as also in *Drosophila* spermatogenesis[25] and blastoderm embryos[26], most ribosomal genes are not transcribed, but a minority are maximally packed with actively transcribing polymerases. If ribosomal gene transcription were regulated by the availability of RNA polymerase molecules, most genes should have had a small number of widely spaced polymerases. The existence of a component which makes a gene open or closed for polymerase attachment is indicated very clearly in our own experiments reported here. By injecting about 10^9 genes into an oocyte, we are increasing by two to three orders of magnitude the endogenous ribosomal gene content of an oocyte nucleus (2×10^6 genes) and by 10^6 the normal content of a somatic nucleus (10^3 genes). Therefore we certainly have an experimental condition in which genes greatly exceed the abundance of their polymerase. We would never expect to see the dense packing of polymerases on a gene (as shown in Fig. 4) in the absence of the postulated gene control component. The fact that we have used cloned genes ensures that all the injected DNA circles which we observe are equally capable of being transcribed. There is otherwise the possibility that some of the untranscribed endogenous genes may be genetically altered and incapable of transcription. Finally, the fact that we inject genes as purified DNA demonstrates particularly clearly that oocytes have available a limited supply of the ribosomal gene-controlling component. This experimental system can now be used to determine whether a similar component affects the transcription of non-ribosomal genes.

Thus, our results indicate that a single ribosomal gene cloned in bacteria can be correctly transcribed after the injection of recombinant plasmid DNA into oocyte nuclei. This encourages the use of injected oocytes to investigate the structure and function of unknown segments of chromosomal DNA which have been cloned in bacteria.

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Sequences from the genome of a non-transforming mutant of polyoma virus

POLYOMA, the small DNA animal tumour virus, like the SV40 virus, has attracted interest because its genome is small enough to be analysed at the molecular level[1–6], enabling study of the molecular basis of oncogenic transformation. In polyoma virus there are several deletion mutants, designated *hr-t* (host-range transformation) mutants, which are unable to transform cells (or cause tumours) and which will grow in certain types of cells only, mainly those which have already been transformed. We report here that we have sequenced the region of the DNA which probably determines the non-transforming phenotype of one such *hr-t* mutant (NG-18)[7] and compared it with the sequence of wild-type polyoma virus DNA. These studies show NG-18 to be a frame-shift mutant, and to have lost 187 of the nucleotides present in wild-type DNA, including several nucleotide triplets which code for cysteine.

NG-18, as well as other *hr-t* mutants, contains a deletion in the 'early region' of the polyoma virus genome[8,9]. This region is thought to contain the coding information for at least three antigenically related proteins, which have been called 'small T-antigen', 'large T-antigen' and membrane-associated or 'middle T-antigen'. The early region probably contains the information required for transformation. The general properties of *hr-t* mutants are as follows: their genetic defect can be rescued[8] by the polyoma virus wild-type restriction fragment *Hpa*II-4, which maps between 78.4 and 91.6 units on the DNA physical map (see Fig. 1); the deletion in NG-18 has been more precisely defined to lie within a *Hae*III subfragment of *Hpa*III-4[8]. A protein the size of small T-antigen (molecular weight 21,000–22,000) is absent from cells infected with *hr-t* mutants[9,10], but a protein indistinguishable in size from the large T-antigen (MW ~ 100,000) is produced[10,11]. *Hr-t* mutants and *ts* *A*-gene mutants (the *A* gene is thought to code for large T-antigen[11]) complement each other for both transformation and virus production[12,13]. Cells infected with *hr-t* mutants also seem to lack a protein (MW ~ 55,000) which occurs in wild-type lytically infected and transformed cells[9,10], is associated with membranes[16] and seems to contain some tryptic peptides in common with small T-antigen[14].

To look in more detail at *hr-t* mutants, we have determined the DNA sequences of the *Hpa*II-4 restriction endonuclease fragment of wild-type polyoma virus DNA (A2 strain)[15] and have compared this with the corresponding DNA sequence found in the *hr-t* mutant, NG-18.

Before sequencing, a fine-structure restriction endonuclease map of *Hpa*II-4 was determined (Fig. 1*b*). Previous analysis of NG-18 DNA with restriction endonucleases showed it to have a deletion of about 3% which mapped at 80–85 units on the physical map of wild-type polyoma virus DNA[4], and to lack the *Sst*I and one of the *Ava*I restriction endonuclease sites found in *Hpa*II-4 (M. Fried, unpublished results). Our studies showed that the fifth largest *Hinf*I restriction endonuclease fragment in polyoma virus DNA contained the NG-18 deletion (Fig. 1*c*) and we used this fragment for sequence analysis across the deletion of the mutant DNA (Fig. 2).

A comparison of sequences found in wild-type polyoma virus DNA (A2 strain) and NG-18 DNA (Fig. 2) gave the following results. (1) Except for the deleted region, the sequences found between 78.4 and 91.6 map units (*Hpa*II-4) in polyoma virus A2 DNA were indistinguishable from those found in the corresponding region of the DNA in NG-18. (2) In NG-18 DNA, 187 base pairs (relative to A2 DNA) were missing, and the deletion could be mapped to lie between 80.5 and 84 units on the physical map. (3) There are many sequences in the region between 79 and 86 map units (Fig. 3) which contain translational termination codons, but these are present in two reading frames only. The third frame contains no termination codons and is presumably used for translation. Following the deletion in NG-18, the sequence does not re-enter the reading frame which has no termination codons, but rather re-enters another reading

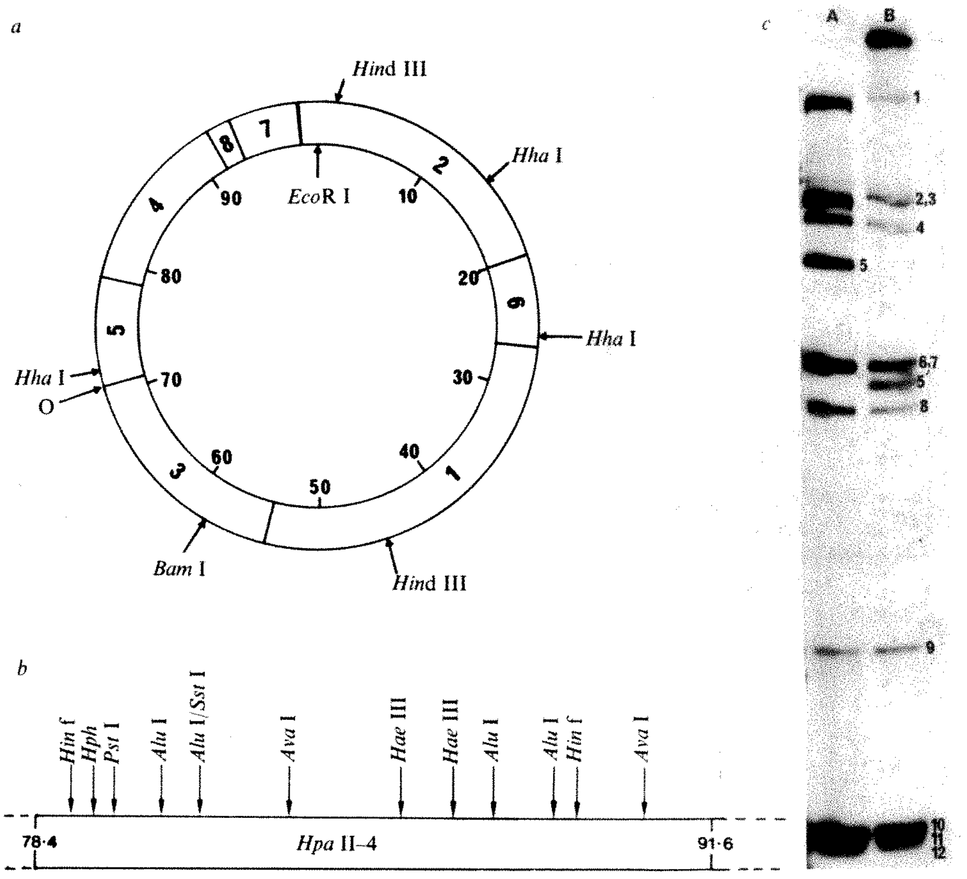


Fig. 1 *a*, The *Hpa*II restriction endonuclease map (100 units) of polyoma virus DNA (A2 strain) showing the region containing the origin of viral replication (O) (taken from ref. 15). *b*, An expanded restriction endonuclease map of the *Hpa*II-4 fragment. *Hpa*II-4 contains the functional deletion found in the *hr-t* mutant, NG-18. The restriction endonuclease sites shown have been independently mapped (ref. 23, Fried, unpublished, and B. E. G., unpublished) and have also been confirmed by sequence analysis (ref. 3 also, see Fig. 3). *c*, An autoradiogram showing A2 (A) and NG-18 (B) DNAs which have been cleaved with the restriction endonuclease *Hinf*I and separated by electrophoresis on a 5% acrylamide–bisacrylamide gel (20× 40 cm)[15]. *Hinf*I cleaves polyoma DNA into 12 fragments, the fifth largest of which is found in *Hpa*II-4 (B. E. G., unpublished). This fragment, which is about 10.5% the size of A2 DNA, occurs as a fragment about 7% in size in NG-18. The NG-18 *Hinf*-7% fragment (band 5' in channel B) has been used to determine the sequences 'missing' in the mutant DNA (see Fig. 2). Cleavage of the ^{32}P-terminally labelled A2 *Hinf*-10.5% fragment with *Hae*III gave new fragments about 7.0 and 2.5% in size; cleavage of NG-18 ^{32}P-terminally labelled *Hinf*-7% gave fragments about 3.5 and 2.5% in size. Sequences from the larger fragment in each case are shown in Fig. 2.

frame, and a termination signal is met within two codons of the deletion. (4) In the 'open' reading frame between 79 and 86 map units, the codon TGT occurs six times and TGC four times in wild-type polyoma DNA. These are cysteine codons. In NG-18 DNA, all but two of these codons are absent.

The data which concern the deletion of NG-18 are summarised in Fig. 3. What conclusions do these data allow? First, as the genetic defect in NG-18 was found to be rescued by the restriction fragment *Hpa*II-4 (ref. 8), and as, except for the deletion found in NG-18, our sequence analysis has shown the mutant and wild-type DNAs to be indistinguishable over this region, it can reasonably be assumed that the deletion is responsible for the mutant phenotype. Second, as NG-18 is essentially a frame-shift mutant, and as the sequences which immediately follow the deletion include a termination codon, any protein(s) induced by the mutant would be smaller than might be expected from a consideration of the size of the deletion alone. Third, the translation product expected from the wild-type DNA would contain a large number of cysteines, whereas a protein from the corresponding region of NG-18 would lack most of these cysteines. If this region of the genome of wild-type virus is coding for a functional protein, the removal of a large number of cysteines would almost certainly alter the conformation of the protein and would be expected to alter its function.

As the large T-antigen proteins made by NG-18 and polyoma wild-type DNA are indistinguishable in size[10,11], it is highly probable that the region of the DNA covered by the deletion is

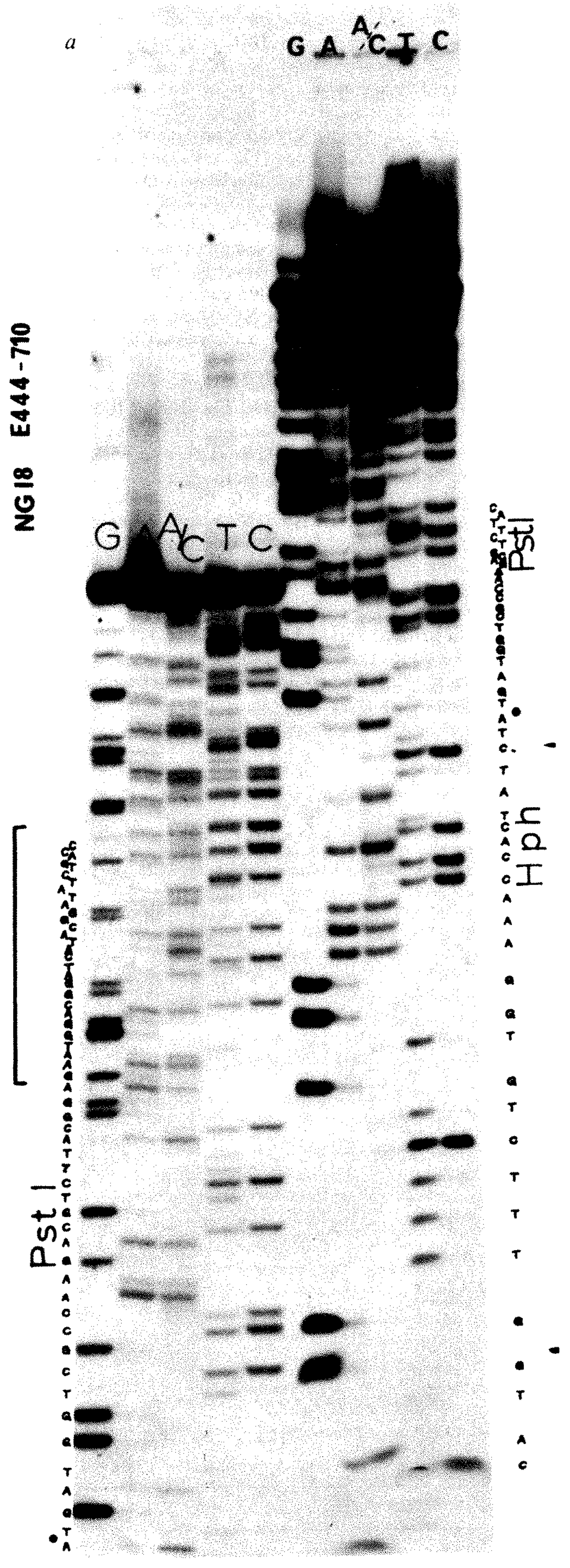
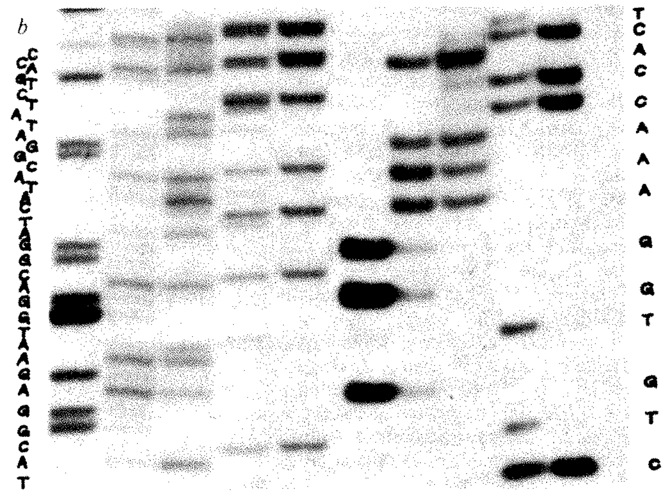


Fig. 2 Polyoma virus DNA and DNA from the *hr-t* mutant NG-18 were cleaved with the restriction endonuclease *Hinf*I. Fragments were labelled at their 3' ends using [α-^{32}P]dATP and the other three cold deoxynucleotide triphosphates (TTP, dCTP and dGTP) in the presence of T4 polymerase[21]. The A2 *Hinf*-10.5% fragment and NG-18-7% fragment (Fig. 1*c*) were isolated, further cleaved with *Hae*III[23], and the sequences of the appropriate fragments (now labelled at one end only) were determined using the techniques described by Maxam and Gilbert[22]. *a*–*c* show autoradiograms of 20% acrylamide–bisacrylamide ges (20 × 40 cm) made essentially as described by Sanger and Coulson[24]; samples were loaded on to the gels at two different time points. The sequence of more than 100 nucleotides can be read from these gels. *a*, The early strand (E) sequence (which contains information complementary to that found in the early mRNAs[25]) from near the *Hinf*I site (79 map units) in NG-18 DNA to a position which corresponds to about 84.3 units on the physical map of polyoma virus DNA (A2 strain) (Fig. 1). (The sequence is underlined by a solid line in Fig. 3.) *b*, An enlargement of the part of *a* which indicates where the deletion occurs in NG-18 (between CAT and ACG in left half of the figure). *c*, The early strand sequences (E) from near the *Hinf*I site in wild-type polyoma virus DNA (A2 strain) to a position five nucleotides beyond the *Sst*I site (GAGCTC) at about 8.6 map units. This sequence is underlined by a dashed line in Fig. 3.



not a coding region for large T-antigen. This implies that in polyoma virus, large T-antigen is either coded for by sequences which occur after 84 map units on the physical map of the DNA (that is, clockwise from 84 map units, see Fig. 1) or large T-antigen is made from a 'spliced' mRNA, such as have been reported for SV40 and adenoviruses[19,20]. The latter argument is supported by several facts: assuming large T-antigen to be entirely virally coded[4], there is not enough coding information left in the 'early region' beyond 84 map units to make a protein the size of large T-antigen. Moreover, among the early mRNAs of polyoma virus, there is an mRNA species that has a splice between 80 and 86 map units; this is postulated to be the mRNA for large T-antigen (R. Kamen, personal communication).

The polypeptide expected from translation of the sequences of polyoma virus DNA found between 79 and 86 map units is shown in Fig. 3*b*. These sequences show some homology (underlined) to sequences predicted to exist in the small T-antigen of SV40 (ref. 18). Although the homology is not extensive, the fact that it exists, that polyoma virus and SV40 seem to share a common overall organisation[4], and that the NG-18 mutant (as well as other *hr-t* mutants) fails to induce small T-antigen, all suggest that sequences which lie between 80.5 and 84 units on the physical map of polyoma virus DNA code for a part of small T-antigen. It seems reasonable to assume, therefore, that small T-antigen may have a rôle in transformation.

c

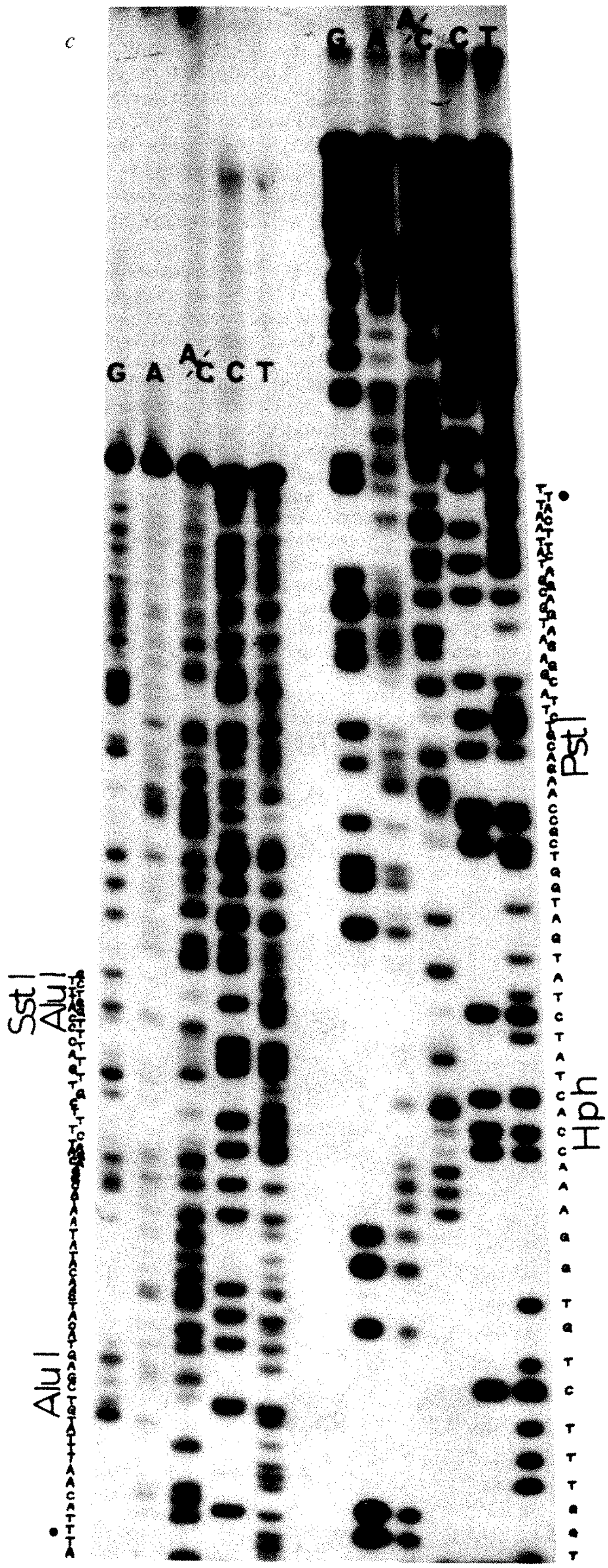


Fig. 3 *a*, Some of the sequencies found in the DNAs of wild-type polyoma virus (A2 strain and the *hr-t* mutant NG-18. DNA sequences designated 'late strand' correspond to sequences found in the early mRNAs[25]. Sequence data for A2 DNA from about 79–86 map units are taken from gels shown in Fig. 2 or from data presented elsewhere[3]. (Numbers shown here and on gels in Fig. 2 represent the number of nucleotides from the *Hpa*II 3–5 junction in polyoma DNA which has been designated the 'zero-point' of the sequence.) NG-18 DNA sequence data are taken in part from the gel shown in Fig. 2. As the parent strain of NG-18 is not known, sequences from other parts of *Hpa*II-4 of mutant DNA were determined, data not given. No differences were seen between mutant and wild-type DNAs. Sequences found in A2 DNA but not in NG-18 DNA are shown within the brackets. Termination codons (TGA, TAG, TAA) are underlined. It can be seen that within this region of the wild-type DNA, only one frame can be used in translation if TGA, TAG and TAA are read as termination codons. The first termination codon found within the 'open' reading frame lies at about 86 map units and is indicated by a box. The protein(s) made from this region of the DNA can be seen to be remarkably cysteine rich (CYS). A protein induced by the NG-18 mutant should lack 8 of the 10 cysteine residues that can be predicted to be found in a protein induced by A2 DNA. *b*, The amino acid composition of a polypeptide predicted by the sequences shown in *a*. Amino acids shown inside the brackets would be absent in a protein translated from NG-18 DNA. Homologous regions between the predicted polypeptide of polyoma virus and small T-antigen of SV40 are underlined[18]; dashed lines show where very similar (ARG/LYS or LEU/ILE) sequences are observed

a

Hinf I (79 map units)

LATE STRAND 5' G AAT CTA AGT ACC AAA GAC ACC TTT GGT GAT AGA TAC TAC CAG CGG

EARLY STRAND 3' C TTA GAT TCA TGG TTT CTG TGT AAA CCA CTA TCT ATG ATG GTC GCC

436 481

CYS CYS CYS CYS

TTC TGC AGA ATG CCT CTT ACC TGC CTA GTA [AAT GTT AAA TAC AGC TCA TGT AGT TGT

AAG ACG TCT TAC GGA GAA TGG ACG GAT CAT [TTA CAA TTT ATG TCG AGT ACA TCA ACA

538

CYS CYS

ATA TTA TGC CTG CTT AGA AAG CAA CAT AGA GAG CTC AAA GAC AAA TGT GAT GCC AGG

TAT AAT ACG GAC GAA TCT TTC GTT GTA TCT CTC GAG TTT CTG TTT ACA CTA CGG TCC

595

CYS CYS CYS CYS

TGC CTA GTA CTT GGA GAA TGT TTT TGT CTT GAA TGT TAC ATG CAA TGG TTT GGA ACA

ACG GAT CAT GAA CCT CTT ACA AAA ACA GAA CTT ACA ATG TAC GTT ACC AAA CCT TGT

652

CCA ACC CGA GAT GTG CTG AAC CTG TAT GCA GAC TTC ATT GCA AGC A]TG CCT ATA GAC

GGT TGG TCT CTA CAC GAC TTG GAC ATA CGT CTA AAG TAA CGT TCG T]AC GGA TAT CTG

709

TGG CTG GAC CTG GAT GTG CAC AGC GTG TAT AAT CCA AGT AAG TAT CAA GAG GGC GGG

ACC GAC CTG GAC CTA CAC GTG TCG CAC ATA TTA GGT TCA TTC ATA GTT CTC CCG CCC

766

(86 map units)

TGG GTA TTT ACG GCC TAT ATT CTT ACA GGG CTC TCC CCC TAG

ACC CAT AAA TGC CGG ATA TAA GAA TGT CCC GAG AGG GGG

805

b

--- ASN LEU SER THR ILE ASP THR PHE GLY ASP ARG TYR TYR GLN ARG

SER CYS ARG MET PRO LEU THR CYS LEU VAL [ASN VAL ILE TYR SER SER CYS SER CYS

ILE LEU CYS LEU LEU ARG LYS GLN HIS ARG GLU LEU LYS ASP CYS CYS ASP ALA ARG

CYS LEU VAL LEU GLY GLU CYS PHE CYS LEU GLU CYS TYR MET GLN TRP PHE GLY THR

PRO THR ARG ASP VAL LEU THR LEU TYR ALA ASP PHE ILE ALA SER] MET PRO ILE ASP

NG18 - CYS LEU TAG

TRP LEU ASP LEU ASP VAL HIS SER VAL TYR ASN PRO SER LYS TRP GLN GLU GLY GLY

TRP VAL PHE THR ALA TRP ILE LEU THR GLY LEU SER PRO TAG

The data obtained from sequencing NG-18 DNA do not allow this role to be assigned exclusively to either the cysteine-rich part of the protein or its normal C terminus, as both should be lacking in a protein induced by NG-18.

For SV40, several viable mutants have been isolated[26] in which deletions occur in a part of the SV40 genome which is comparable to the region in polyoma virus genome where the *hr-t* mutants have been mapped. These mutants fail to induce a protein the size of small T-antigen of SV40 (ref. 27) but do induce 'new' smaller proteins. Although the deletions in the SV40 mutants seem to have an effect on transformation, unlike the deletions in polyoma virus *hr-t* mutants, they do not entirely inhibit it[28,29]. Further studies on other polyoma *hr-t* mutants and the SV40 mutants may therefore provide some insight into transformation.

One further point remains to be considered. No protein comparable to the membrane-associated (MW ~ 55,000) protein reported for polyoma virus[16] has been found for SV40. As NG-18 also fails to induce this protein, it may also be coded for by sequences which lie between 80.5 and 84 map units on the physical map of polyoma virus DNA, and may also play a part in transformation. One of the next problems for study will be to determine whether the phenotypic differences between the polyoma virus and SV40 mutants are associated with small T-antigen or the membrane-associated protein, or possibly with both.

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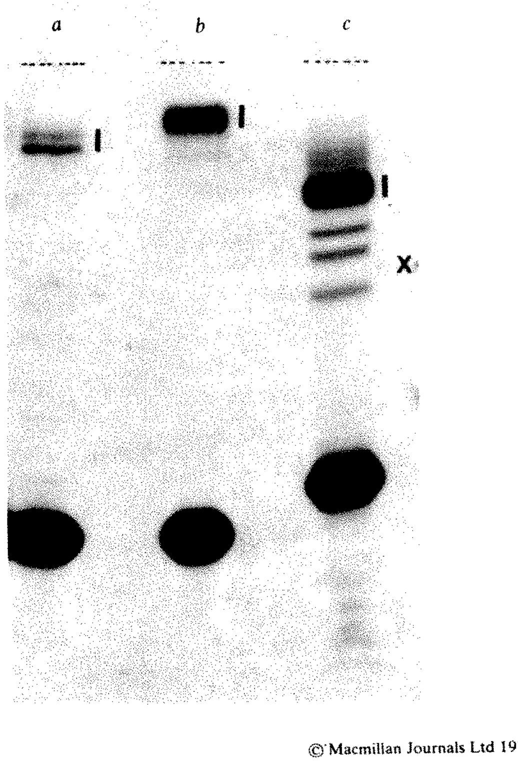
3′-Terminal nucleotide sequences in the genome RNA of picornaviruses

IN common with eukaryotic cellular mRNAs[1–3], the terminal sequences in virus RNAs contain untranslated regions of variable length[4–11]. These regions are likely to contain binding sites for macromolecules involved in functions such as replication[4] and translation[4–6] of the RNA, and assembly of virus particles. We report here that, as a first step in identifying these sites we have sequenced between 33 and 156 nucleotides next to the 3′-terminal poly(A)[12–14] of representative viruses from three of the four picornavirus genera[15], the enteroviruses, cardioviruses and foot-and-mouth disease viruses (FMDV). The viruses chosen for these analyses allow us to make comparisons of the 3′-terminal sequences both within and between the three genera. Within each genus the 3′-terminal sequences are at least 60% homologous, whereas viruses from different genera show little similarity. The 3′-terminal sequences also show some unexpected features, including the lack of the putative signal sequence 5′-AAUAAA-3′ (ref. 1) in viruses from two genera.

The virus RNA was partially[16] copied into complementary DNA (cDNA) from the poly(A) with an oligo(dT)-containing primer and DNA polymerase I as described previously[17], except that the cDNA was further purified (Fig. 1) so as to select only the larger DNA copies. These copies, not less than 43 nucleotides in length (primer included), were sequenced using the rapid

Fig. 1 Gel electrophoresis of [5′-^{32}P] complementary DNA synthesised with the RNA of *a*, SVDV; *b*, poliovirus; *c*, FMDV, type SAT 2. The cDNA was synthesised in an incubation[17] (1 h at 37 °C) containing: virus RNA (2–20 μg) purified as described elsewhere[32], Tris-HCl *p*H 7.5 (67 mM), $MnCl_2$ (0.5 mM), β-mercaptoethanol (0.13 mM), NaCl (0.16 mM), [5′-^{32}P] $p(dT)_{10}dG$ or [5′-^{32}P] $p(dT)_8dC$ (~100 pmol) purified as described previously[17], all four unlabelled deoxytriphosphates (each 0.67 mM), actinomycin D (50 μg ml^{-1}) and DNA polymerase I, Klenow fragment (4–8 units). Subsequently, the cDNA was isolated by phenol/chloroform extraction and ethanol precipitation. The transcription products were then separated by electrophoresis for 2–3 h at 900 V in a 15% polyacrylamide (0.6% bisacrylamide) gel (20 × 40 cm) containing 50 mM Tris-borate, 1 mM EDTA and 7 M urea, as shown. The bands migrating more slowly than the xylene–cyanol FF marker (×), indicated by a bar, were visualised by autoradiography and the cDNA eluted into water by homogenisation (4 extractions), then precipitated in a volume of 3.0 ml containing 0.05 M NaAc *p*H 5.4, 60 μg carrier RNA and 75% ethanol. The intense, fast migrating bands at the bottom of the diagram contain unincorporated primer, [5′-^{32}P] $p(dT_8)dC$ in lanes *a* and *b*, and [5′-^{32}P] $p(dT)_{10}dG$ in lane *c*.



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chemical sequencing technique for [5′-^{32}P]DNA (ref. 18). Figure 2 shows polyacrylamide gels displaying sequences complementary to the 3′-terminal regions of several picornavirus RNAs. The clarity of the band patterns indicates that transcription occurred exclusively from the poly(A), and allows 3′-terminal sequences to be read without difficulty. The 3′-terminal sequence of encephalomyocarditis virus (EMCV) RNA has previously[17] been determined up to position 129 by the chemical method, and also in part by independent DNA and RNA sequencing techniques which indicated that the chemical method is reliable. In the present study, the chemically determined 3′-terminal sequences of poliovirus RNA (type 1, Mahoney strain) and FMDV RNA (type A, strain 61) have also been checked by other methods (not shown). In the case of poliovirus RNA, the sequence between positions 10 and 39 has been verified by the dideoxynucleotide method[19], with reverse transcriptase as the copying enzyme and a dideoxynucleotide to deoxynucleotide ratio of 2.5:1. The 3′-terminal sequence of 20 nucleotides in the FMDV RNA was verified using the 'plus–minus' method[20,21].

The few nucleotide sequence uncertainties were, as expected, near the 3′-end of the cDNA (Fig. 3), where gel resolution was not perfect. One anomaly was observed in poliovirus cDNA (not shown) in that at nucleotide 92, a dG and a dC apparently occur in the same position. This may be due either to anomalous gel mobility caused by strong secondary structure[19] or to heterogeneity in nucleotide sequence in one position[22].

The 3′-terminal sequences for nine picornaviruses, determined in at least two independent experiments, are shown in Fig. 3. In all polyadenylated RNAs previously examined[1,3,23,24] (except possibly Rous sarcoma virus RNA[25]), the sequence 5′-AAUAAA-3′ is located in a similar position in the untranslated region near the poly(A). This sequence has been implicated as a signal in polyadenylation[17] as it is absent from plant virus RNAs, which are not polyadenylated[8]. The same A-rich hexanucleotide is also present in EMCV (ref. 10) and maus-Elberfeld virus (MEV) RNAs near the poly(A), but is absent from the 3′-terminal region in the two enterovirus and five FMDV RNAs examined here (Fig. 3). (Although GAUAAA is present in swine vesicular disease virus around position 40, its presence must be considered fortuitous, as it is located much further from where AAUAAA is normally found[1–3], and is absent from the corresponding region of poliovirus RNA.) The absence of an AAUAAA sequence near the 3′-end in the RNAs of the enteroviruses and FMDVs, all of which are polyadenylated, therefore brings into doubt the suggestion[17] that this A-rich sequence is a universal signal for polyadenylation.

In the cardioviruses, a cluster of seven nonsense triplets occurs near the poly(A), with a frequency much greater than would be expected in a random sequence. At least two nonsense triplets block all three protein synthesis reading frames[10], suggesting this as a possible device for preventing protein synthesis continuing into the poly(A) as a result of a frameshift mutation, or suppression of the natural termination codon of the coding region. Nonsense triplets are almost as numerous in the enteroviruses, though more widely spaced. As with the cardioviruses, they occur in the three possible reading frames, but with only two of the frames blocked more than once. Inspection of the positions of the nonsense triplets in all the picornavirus RNAs allows the minimum number of untranslated nucleotides next to the poly(A) to be deduced. The highest number so far (43) is in serotype SAT 2 of FMDV.

Unexpectedly, we found that two classes of cDNA are synthesised with the RNA of the C997 strain of FMDV (Fig. 2). The larger cDNA (C997(L)) is ~60–70 nucleotides long and is very similar in sequence to the p(dT)$_{10}$dG-primed cDNAs from four other FMDV RNAs (Fig. 3). The smaller cDNA (C997(S)) is ~43–53 nucleotides long and is identical in sequence to C997(L) in the 20 nucleotides next to the oligo(dT) primer; then, in complete contrast to C997(L), there is a run of at least 13 dA residues, giving a total of 17 consecutive dA residues (Fig. 2). Therefore, there seems to be an oligo(U) tract in some C997 virus RNA molecules near the poly(A). An alternative explanation is that the oligo(dA) is an artefact resulting from slippage of the DNA polymerase on reaching a short run of U residues in the template, but this is considered unlikely, for once DNA synthesis has been initiated, slippage is not known to occur with DNA polymerase. It is also unlikely that slippage would occur on only some template molecules. Preliminary RNase T$_1$ two-dimensional fingerprinting experiments with C997 virus RNA have shown the presence of a spot, the position of which would agree with both the size and composition of the oligo(U) tract detected in the sequencing studies (D.N.B. and K. J. H. Robson, unpublished data).

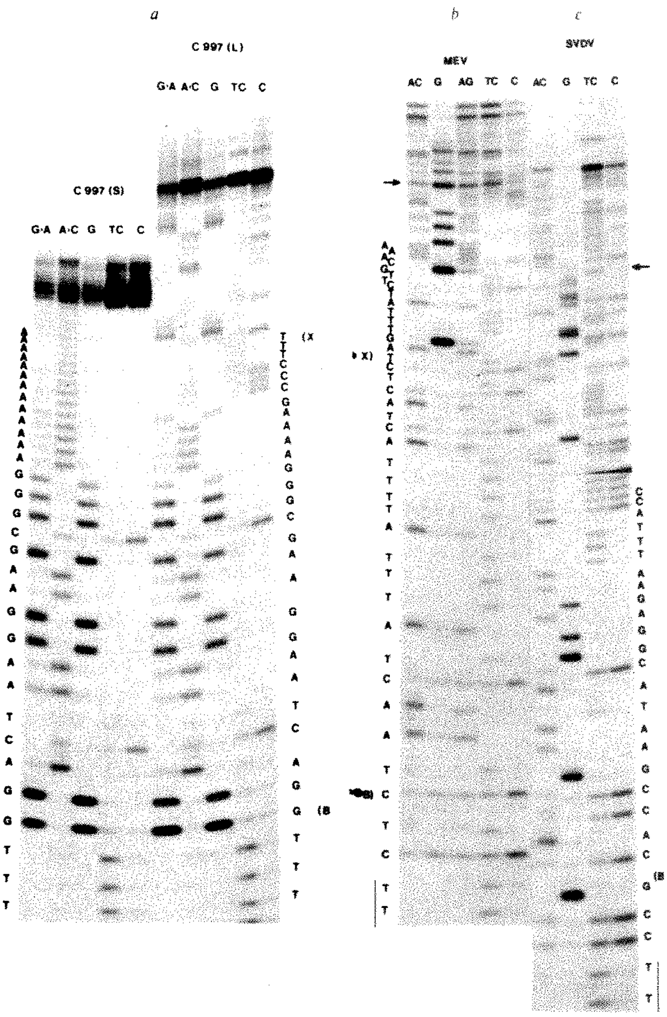

Fig. 2 Sequencing gels of *a*, [5′-^{32}P] p(dT)$_{10}$dG-primed cDNA synthesised with FMDV RNA, strain C997; *b*, [5′-^{32}P] p(dT)$_8$dC-primed cDNA from maus-Elberfeld virus RNA; *c*, [5′-^{32}P] p(T)$_8$dC-primed cDNA from SVDV RNA. The [5′-^{32}P] cDNA eluted from the polyacrylamide gel (Fig. 1) was divided into four or five equal volumes and each aliquot subjected to one of the six base-specific partial chemical degradations[18] (dG > dA, dA + dG, dA > dC, dG, dT + dC or dC). The gel electrophoretic separation of the partial products was carried out as described[18], with the following slight modifications: the gel (30 × 80 cm) was the same composition as that shown in Fig. 1; the samples were loaded in 5 mM Trisborate, 7 M urea, 0.1 mM EDTA, 0.05% bromphenol blue and xylene–cyanol FF, and 20% sucrose; the top buffer compartment contained 7 M urea; the gel was pre-run for 16 H at 1 kV, and electrophoresis was for 24–48 h at 1.3–1.8 kV. Autoradiography was for 7–21 d at −20 °C. The prefix d for deoxy has been omitted.

Oligo- or poly(U) has not been found previously in the messenger strand of any virus, although it seems to be present at the 5′ end of the poliovirus minus strand[26], where it functions as the complement of the poly(A) on the opposite (plus) strand. It is not known whether oligo(U) has a special function in C997, nor whether it is present in other C serotypes. We have not detected

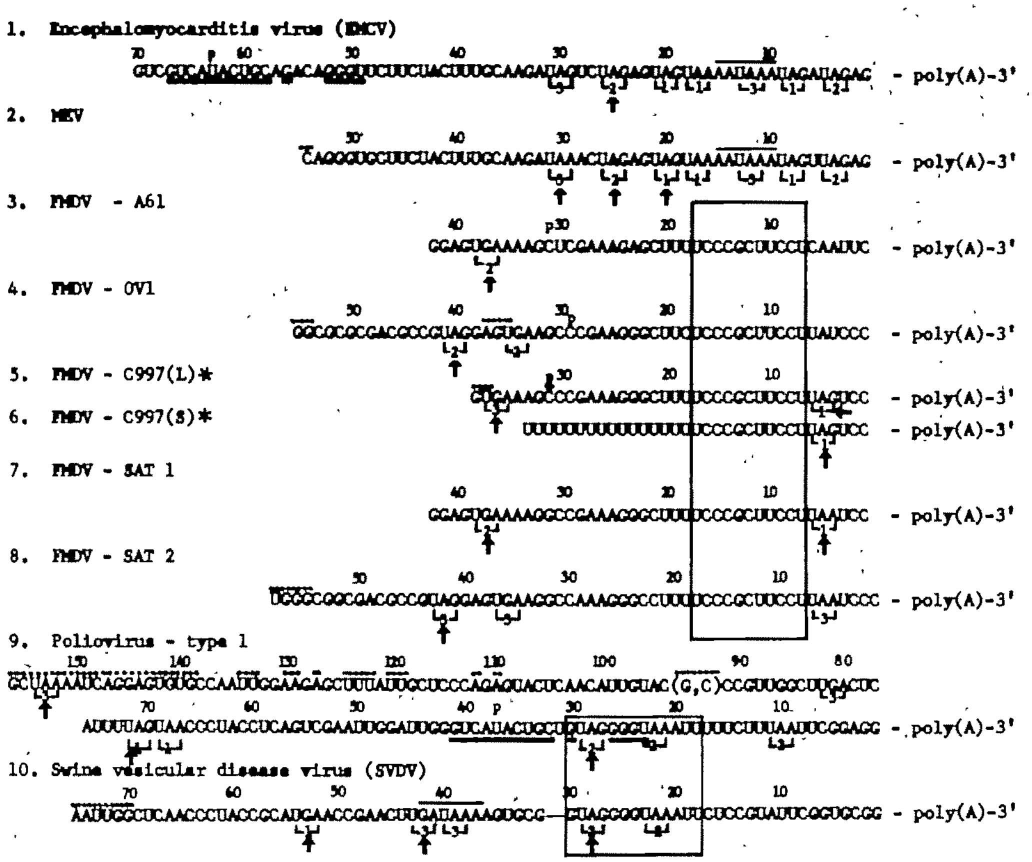

Fig. 3 3'-Terminal sequences of nine picornavirus RNAs. The sequences were deduced from the band patterns on the gels (Fig 2). Slight uncertainties are indicated by a dotted line. In the enteroviruses and FMDVs, the highly conserved areas are boxed in. A thick line draws attention to the homology between poliovirus and EMCV RNA. A thin line shows the sequences AAUAAA and GAUAAA. The letter p indicates palindromic sequences. Nonsense triplets are bracketed, and the protein synthesis reading frames in which they occur are indicated. Arrows point to potential termination codons of the coding region. Note that in EMCV the single potential termination codon was assigned from the positions of the other nonsense triplets in the sequence of 129 nucleotides next to the poly(A) (ref. 17). *L and S refer to sequence derived from large or small cDNA

oligo(dA) in the cDNA transcribed from the RNAs of four other FMDV serotypes, although the possibility of its presence in poorly transcribed cDNA cannot be ruled out.

There is considerable conservation of 3'-terminal sequences within the cardiovirus and FMDV groups (Fig. 3). The two cardioviruses are $>90\%$ homologous in the 54 nucleotides next to the poly(A). The five FMDV serotypes are $\sim75\%$ homologous in the 20 nucleotides next to the poly(A), and this includes a highly conserved sequence of 11 consecutive nucleotides, which is indicated in Fig. 3. Excluding the RNA corresponding to C997(S) cDNA, all five serotypes of FMDV also show $\sim75\%$ homology in the 38 nucleotides next to the poly(A). In this region, a purine-rich sequence (20 nucleotides) precedes a pyrimidine-rich sequence (20 nucleotides), suggesting that stable secondary structure could exist. For example, in the A61 strain, nucleotides 16–24 paired with nucleotides 29–37 would give a thermodynamically stable structure ($\Delta G \approx -8.4$ kcal mol^{-1}) (ref. 27). No such structure is likely in the C997 RNA species containing oligo(U), in which the 3'-terminal 33 nucleotides are almost entirely pyrimidines. There is, however, an opportunity for an alternative secondary structure in this RNA in which the oligo(U) pairs with the poly(A). The serologically unrelated enteroviruses, poliovirus and swine vesicular disease virus, are $>60\%$ homologous in the 71 nucleotides adjacent to the poly(A). Figure 3 draws attention to a highly conserved region in these virus RNAs. In SVDV, nucleotides 25–35 could pair with nucleotides 54–64, giving a highly stable double-stranded region[27] ($\Delta G \approx 12.0$ kcal mol^{-1}) in which seven out of 11 nucleotide pairs are GC. Only a marginally stable secondary structure could exist, however, involving the corresponding sections of poliovirus RNA.

Homologies between viruses from different genera are much less frequent. Figure 3 indicates one significant homology between poliovirus and EMCV, in which 15 out of 19 nucleotides are identical, between positions 49 and 67 in EMCV and positions 23 and 41 in poliovirus. It is interesting that much of the homologous region has the same nucleotide sequence when read in either direction[17] (a palindrome). 11- or 13-nucleotide palindromes are also present in the RNAs of the FMDV strains A61, OV1 and C997, centred at or near position 30. Palindromes have been found previously in the 3'-untranslated regions of host mRNAs from several different sources[24,28–30], and it has been suggested that they may provide binding sites for oligomeric proteins in the corresponding DNA[31]. However, this cannot be the case for the picornaviruses, which do not go through a DNA intermediate in their replication.

In conclusion, the presence of many nonsense triplets near the 3' terminus of some picornavirus RNAs, the absence of the putative signal AAUAAA in viruses from two genera and evidence of oligo(U) in a fraction of RNA from one virus, are all unexpected findings in the picornavirus genome. As expected, at least part of the 3'-terminal region in all picornavirus RNAs so far studied is not expressed into protein. It is possible that this untranslated portion has a special function, for example, to bind the virus polymerase complex during initiation of replication. Support for the idea that the 3' end may be specifically recognised comes from our finding that extensive sequence homologies exist in the RNAs of viruses within each picornavirus genus, whereas viruses from different genera show little similarity. These findings also strongly support the accepted scheme[15] for the division of picornaviruses into genera.

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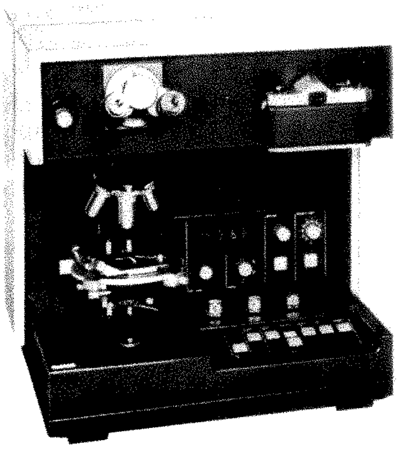

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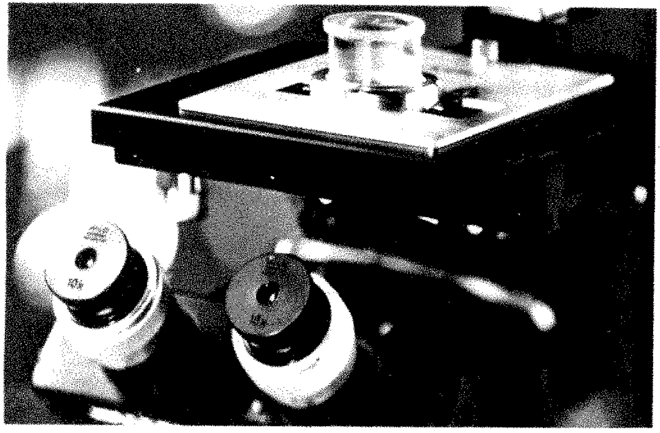

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DNA sequence of the bacteriophage λ *c*I gene

THE *c*I gene of bacteriophage λ codes for the λ repressor. This protein is both a positive and a negative regulator of gene transcription (see ref. 1 for review). These functions are mediated by the interaction of repressor with two operators in the phage DNA. Both the nucleotide sequence of the λ operators[2-5] and the amino acid sequence of the λ repressor have been determined[6]. The ability of the λ repressor to bind operator DNA is greatly reduced by a proteolytic cleavage which occurs, for example, during *recA*-dependent prophage induction in *Escherichia coli*[7]. A single mutation (λ*c*I *ind*$^-$) prevents this cleavage and inactivation of repressor[7,8]. I present here the nucleotide sequence of the λ *c*I gene and identify the base change responsible for the ind$^-$ phenotype of λ repressor.

Restriction endonuclease fragments containing *c*I DNA were isolated either from phage λ DNA or from the plasmid pKB252 (ref. 9) by gel electrophoresis[10], and were end-labelled and sequenced by the chemical method of Maxam and Gilbert[11]. A partial restriction map of *c*I and surrounding DNA, and the positions and strands of sequenced restriction fragments are shown schematically in Fig. 1. Restriction fragments have been aligned by direct DNA sequence overlaps. The nucleotide sequence of *c*I and the amino acid sequence of the λ repressor[6] are shown in Fig. 2.

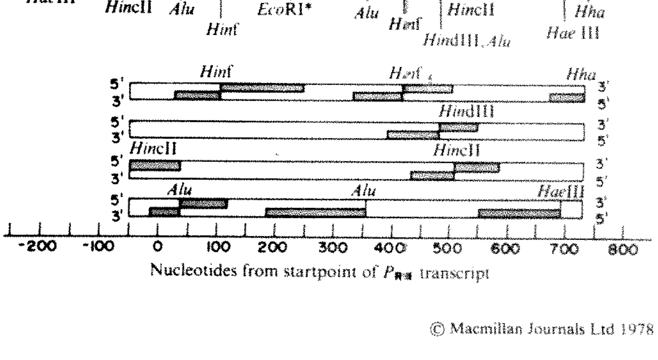
The sequence 5′ CCAGG 3′ occurs twice in the sense strand of *c*I DNA (bases 99–103 and 529–533) The second cytosine in this sequence is normally present as 5-methylcytosine in wild-type strains of *E. coli*[12,13]. As 5-methylcytosine fails to give a band in the chemical DNA sequencing method[11,14], its location is usually deduced by the presence of a characteristic gap in the pattern of bands, combined with sequence analysis of the complementary DNA strand. The presence of 5-methylcytosine at base 100 of the *c*I sequence was confirmed by these methods. The assignment of base 530 as 5-methylcytosine relies on a comparison of the protein and DNA sequences. The DNA sequence read from the sequencing gel was 5′ GAG C_{530}A GGT 3′, whereas the corresponding λ repressor sequence is Glu^{175}-Pro^{176}-Gly^{177}. The genetic code requires that proline be coded by a CCN codon, and therefore I assume that base 530 is present as 5-methylcytosine. Bases 102 and 532 of the *c*I antisense sequence are also present as 5-methylcytosines. In the *lacI* gene, 5-methylcytosines have been identified as hot spots for spontaneous and 2-aminopurine induced mutagenesis[15].

The DNA sequence of *c*I (Fig. 2) confirms the amino acid sequence determined for λ repressor by independent protein-sequencing methods[6]. The nucleotide sequence also agrees with the partial sequences determined previously for the 5′ and 3′ termini of *c*I[4,5,16] and for the 5′ sequence of *c*I mRNA transcribed from the P_{RM} promoter[1,16].

The *ind*$^-$ *c*I mutation was sequenced from the *Hinf*/*Eco*RI* 199-base-pair fragment (Fig. 1) isolated from phage λ DNA carrying the mutant allele. *ind*$^-$ *c*I DNA has an A at base 352 replacing the wild-type G. This base change generates an additional *Hin*dIII recognition site at bases 352–357 of *c*I. The presence of this additional restriction site in λ*c*I *ind*$^-$ DNA has been noted elsewhere[17]. The *ind*$^-$ base change occurs approximately halfway through *c*I, a position which correlates extremely well with its location in the genetic map constructed by Lieb[18]. In *ind*$^-$ λ repressor, a lysine should replace the wild-type glutamic acid at amino acid 117. The mutant repressor is thus considerably more basic than the wild type in physiological conditions.

The codon usage for wild-type *c*I is given in Fig. 3. Overall, there is a slight preference for T and A in the third base of the codons. However, the third position distribution is biased by the 11 codons in which G is obligatory (ATG and TGG) and the 50 codons for Lys, Glu and Gln which require either an A or G in the third position. In those triplets where any of the four bases is acceptable (CTN, GTN, TCN, CCN, ACN, GCN, CGN and GGN) there is a marked preference for T (38.6%) and a discrimination against G (10.9%) in the third position.

Fig. 1 Partial restriction map of *c*I and surrounding DNA. Restriction sites used for DNA sequence analysis of *c*I are indicated. Not all sites for *Eco*RI*, *Alu* and *Hha* are shown. The hatched areas in the lower scheme indicate the extent of reliable DNA sequence information from particular restriction cuts. Restriction fragments were 5′ labelled with [γ-^{32}P]ATP and polynucleotide kinase[11], and were cut with a second restriction enzyme. All sequencing is thus 5′→3′. The upper strand is the *c*I sense strand.



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NH2-Ser-Thr-Lys-Lys-Lys-Pro-Leu-Thr-Gln-Glu-Gln-Leu-Glu-Asp-Ala-Arg-Arg-Leu-Lys-Ala-
ATG AGC ACA AAA AAG AAA CCA TTA ACA CAA GAG CAG CTT GAG GAC GCA CGT CGC CTT AAA GCA
TAC TCG TGT TTT TTC TTT GGT AAT TGT GTT CTC GTC GAA CTC CTG CGT GCA GCG GAA TTT CGT
             10          20          30              40          50          60

    Ile-Tyr-Glu-Lys-Lys-Lys-Asn-Glu-Leu-Gly-Leu-Ser-Gln-Glu-Ser-Val-Ala-Asp-Lys-Met-
    ATT TAT GAA AAA AAG AAA AAT GAA CTT GGC TTA TCC CAG GAA TCT GTC GCA GAC AAG ATG
    TAA ATA CTT TTT TTC TTT TTA CTT GAA CCG AAT AGG GTC CTT AGA CAG CGT CTG TTC TAC
             70          80          90              100         110         120

    Gly-Met-Gly-Gln-Ser-Gly-Val-Gly-Ala-Leu-Phe-Asn-Gly-Ile-Asn-Ala-Leu-Asn-Ala-Tyr-
    GGG ATG GGG CAG TCA GGC GTT GGT GCT TTA TTT AAT GGC ATC AAT GCA TTA AAT GCT TAT
    CCC TAC CCC GTC AGT CCG CAA CCA CGA AAT AAA TTA CCG TAG TTA CGT AAT TTA CGA ATA
             130         140         150             160         170         180

    Asn-Ala-Ala-Leu-Leu-Ala-Lys-Ile-Leu-Lys-Val-Ser-Val-Glu-Glu-Phe-Ser-Pro-Ser-Ile-
    AAC GCC GCA TTG CTT GCA AAA ATT CTC AAA GTT AGC GTT GAA GAA TTT AGC CCT TCA ATC
    TTG CGG CGT AAC GAA CGT TTT TAA GAG TTT CAA TCG CAA CTT CTT AAA TCG GGA AGT TAG
             190         200         210             220         230         240

    Ala-Arg-Glu-Ile-Tyr-Glu-Met-Tyr-Glu-Ala-Val-Ser-Met-Gln-Pro-Ser-Leu-Arg-Ser-Glu-
    GCC AGA GAA ATC TAC GAG ATG TAT GAA GCG GTT AGT ATG CAG CCG TCA CTT AGA AGT GAG
    CGG TCT CTT TAG ATG CTC TAC ATA CTT CGC CAA TCA TAC GTC GGC AGT GAA TCT TCA CTC
             250         260         270             280         290         300

    Tyr-Glu-Tyr-Pro-Val-Phe-Ser-His-Val-Gln-Ala-Gly-Met-Phe-Ser-Pro-Glu-Leu-Arg-Thr-
    TAT GAG TAC CCT GTT TTT TCT CAT GTT CAG GCA GGG ATG TTC TCA CCT GAG CTT AGA ACC
    ATA CTC ATG GGA CAA AAA AGA GTA CAA GTC CGT CCC TAC AAG AGT GGA CTC GAA TCT TGG
             310         320         330             340         350         360

    Phe-Thr-Lys-Gly-Asp-Ala-Glu-Arg-Trp-Val-Ser-Thr-Thr-Lys-Lys-Ala-Ser-Asp-Ser-Ala-
    TTT ACC AAA GGT GAT GCG GAG AGA TGG GTA AGC ACA ACC AAA AAA GCC AGT GAT TCT GCA
    AAA TGG TTT CCA CTA CGC CTC TCT ACC CAT TCG TGT TGG TTT TTT CGG TCA CTA AGA CGT
             370         380         390             400         410         420

    Phe-Trp-Leu-Glu-Val-Glu-Gly-Asn-Ser-Met-Thr-Ala-Pro-Thr-Gly-Ser-Lys-Pro-Ser-Phe-
    TTC TGG CTT GAG GTT GAA GGT AAT TCC ATG ACC GCA CCA ACA GGC TCC AAG CCA AGC TTT
    AAG ACC GAA CTC CAA CTT CCA TTA AGG TAC TGG CGT GGT TGT CCG AGG TTC GGT TCG AAA
             430         440         450             460         470         480

    Pro-Asp-Gly-Met-Leu-Ile-Leu-Val-Asp-Pro-Glu-Gln-Ala-Val-Glu-Pro-Gly-Asp-Phe-Cys-
    CCT GAC GGA ATG TTA ATT CTC GTT GAC CCT GAG CAG GCT GTT GAG CCA GGT GAT TTC TGC
    GGA CTG CCT TAC AAT TAA GAG CAA CTG GGA CTC GTC CGA CAA CTC GGT CCA CTA AAG ACG
             490         500         510             520         530         540

    Ile-Ala-Arg-Leu-Gly-Gly-Asp-Glu-Phe-Thr-Phe-Lys-Lys-Leu-Ile-Arg-Asp-Ser-Gly-Gln-
    ATA GCC AGA CTT GGG GGT GAT GAG TTT ACC TTC AAG AAA CTG ATC AGG GAT AGC GGT CAG
    TAT CGG TCT GAA CCC CCA CTA CTC AAA TGG AAG TTC TTT GAC TAG TCC CTA TCG CCA GTC
             550         560         570             580         590         600

    Val-Phe-Leu-Gln-Pro-Leu-Asn-Pro-Gln-Tyr-Pro-Met-Ile-Pro-Cys-Asn-Glu-Ser-Cys-Ser-
    GTG TTT TTA CAA CCA CTA AAC CCA CAG TAC CCA ATG ATC CCA TGC AAT GAG AGT TGT TCC
    CAC AAA AAT GTT GGT GAT TTG GGT GTC ATG GGT TAC TAG GGT ACG TTA CTC TCA ACA AGG
             610         620         630             640         650         660

    Val-Val-Gly-Lys-Val-Ile-Ala-Ser-Gln-Trp-Pro-Glu-Glu-Thr-Phe-Gly-COOH
    GTT GTG GGG AAA GTT ATC GCT AGT CAG TGG CCT GAA GAG ACG TTT GGC TGA TCG
    CAA CAC CCC TTT CAA TAG CGA TCA GTC ACC GGA CTT CTC TGC AAA CCG ACT AGC
             670         680         690             700         710
```

Fig. 2 DNA sequence of *c*I and amino acid sequence of λ repressor. The subscripted numbers represent the number of bases from the start of the *c*I P_{RM} transcript. Cytosines at bases 100 and 530 of the sense sequence, and 102 and 532 of the antisense sequence are present as 5-methylcytosines (see text).

FIRST BASE	SECOND BASE T	C	A	G	THIRD BASE
T	PHE 8	SER 3	TYR 4	CYS 1	T
	PHE 4	SER 4	TYR 3	CYS 2	C
	LEU 6	SER 4	STOP 0	STOP 1	A
	LEU 1	SER 0	STOP 0	TRP 3	G
C	LEU 8	PRO 6	HIS 1	ARG 1	T
	LEU 2	PRO 0	HIS 0	ARG 1	C
	LEU 1	PRO 8	GLN 2	ARG 0	A
	LEU 1	PRO 1	GLN 10	ARG 0	G
A	ILE 3	THR 0	ASN 6	SER 5	T
	ILE 6	THR 5	ASN 2	SER 6	C
	ILE 1	THR 4	LYS 12	ARG 5	A
	MET 8	THR 1	LYS 5	ARG 1	G
G	VAL 11	ALA 4	ASP 5	GLY 6	T
	VAL 1	ALA 5	ASP 4	GLY 5	C
	VAL 1	ALA 9	GLU 9	GLY 1	A
	VAL 2	ALA 1	GLU 12	GLY 5	G

Fig. 3 Codon usage for wild-type *c*I The overall third position base frequencies are T, 30.5%; C, 21.1%; A, 27.1%; G, 21.1%. In those codons where any of the four bases in the third position specify the same amino acid (see text) the frequencies are T, 38.6%; C, 22.7%; A, 27 7%; G, 10.9%.

I thank Allan Maxam and Keith Backman for advice and discussion, Alexander Johnson for some of the *c*I restriction fragments, and Mark Ptashne for support. This work was supported by grants from the NIH and the NSF.

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reviews

New ecology exhibition at BM

Peter D. Moore

Introducing Ecology. British Museum (Natural History) Exhibition. *Nature at Work.* By British Museum (Natural History). Pp. 96. (Cambridge University Press. Cambridge, 1978.) Hardback £6.50; paperback £2.50.

AFTER about fifty years of conceptual evolution, the idea of the ecosystem, involving close relationships between the living and non-living world, is finally having an impact at the level of elementary biology teaching This is very welcome, for it may result in a more precise understanding of the fundamental basis of modern ecology and restore some meaning to the very term. The exhibition recently launched at the British Museum (Natural History), together with the associated book, will certainly serve to encourage this laudable trend.

The exhibition and the book concentrate on the energy relationships of ecosystems, which has the advantage of providing a unifying theme for the concepts portrayed but which inevitably results in the neglect of other interesting aspects of ecosystem study. Unity and flow, however, are vital if the interest of a visitor is to be maintained and this is certainly achieved. But energy is not easily conceived, an early display tries to overcome this by encouraging the observer to turn a wheel on the exhibit with sufficient gusto to generate the electricity for lighting the panels. Great fun for young children, but I wonder whether it really helps with their thermodynamics?

Energy movement through ecosystems as a theme also generates the problem of how to explain photosynthesis, the primary level of energy conversion. Here the exhibition and the book are at their weakest. and understandably so. One is left with two options: either you try to explain the complex mechanism of photophosphorylation or you leave photosynthesis as a black box with sunlight going in and energy-rich foodstuffs coming out. In fact the former course was chosen. Chloroplast structure is explained in some detail and the production of ATP and NADH is mentioned. but chlorophyll itself remains something of a black box. It is rather strange that this degree of biochemical detail is used at this stage when no equivalent account is given of respiration, a process just as vital to an understanding of energy flow. Also, as the emphasis throughout is on the presentation of concepts in a simple, pictorial fashion, is there any need to introduce such technical terms as autotrophs, heterotrophs, or even carbohydrates? Many of the weaker brethren my stumble needlessly at this first hurdle.

One of the unfortunate consequences of the energy flow concept from the botanical point of view is that the plant is reduced to the level of an energy conversion and storage system on which the rest of the (far more interesting) organisms depend. When photosynthesis has been dealt with, plants can be set on one side. Admittedly, some attempt is made to show the influence of climate and soil conditions on plant growth, but this display is difficult to follow because of its complexity.

From this point onwards, the quality of both exhibition and book begin to rise. The ways in which consumer organisms make a living are described and attention is drawn to the energy which their way of life demands in such activities as seeking food. Two habitats are considered in detail: an oak woodland (in which some taxonomic confusion over the species of oak involved is evident) and a rocky seashore (in which *Fucus serratus* is found above *Pelvetia canaliculata*). In dioramas of these habitats, the student is presented with such problems as working out food webs. In the book the answers are supplied at the end, but in the exhibition electronic links can be made between different organisms which results in a red or green light appearing, depending on the likelihood of one consuming the other. Observations on secondary school children operating this display suggested that many were unaware of the implications of the red or green light but this, I suppose, its one of the problems which educationists have to face.

Decomposition is tackled in a novel and effective way using animated flicker cartoons which are operated by the observer. The consequent recycling of elements is not fully dealth with at present, but I gather that a display of the carbon cycle will be added to the exhibition later.

Perhaps the most ambitious and, in my view, the most successful aspect of energy flow to be considered is the energy pyramid and ecological efficiency. Those students who have worked their way this far without losing the thread are presented with such fascinating questions as why carnivores may be more efficient than herbivores, and why the pyramid of biomass in aquatic systems is an inversion of that found on land.

Watching children in the exhibition one soon comes to the conclusion that the most popular exhibits are those with which they can interact, where questions are asked, choices made and buttons pressed. In this respect a new dimension is attained at this exhibition. Having completed the exhibition circuit, the student meets a computer console and screen with which he can tackle an ecological question. A problem is set up by the computer, such as what factors control winter moth caterpillars in oakwoods. Several alternative solutions are presented and the operator makes a choice which results in the presentation of data either supporting or negating the selection. Rejection of that hypothesis then returns the student to the initial choices The game provides a unique opportunity for a student to work his way through an ecological problem in a systematic and scientific fashion

Presentation of material in both exhibition and book is clear, graphic and caricatured, sometimes to the extent of being bizarre This will offend the purist But whatever the dusty academic may think of this venture, the fact remains that on the one hand a seven year old child may find the demonstrations rivetting in their overt appeal and on the other there are questions raised here which still occupy the minds of research workers. The potential spectrum of interest is vast and the message is clear and basic, that ecology has moved beyond natural history and has become a unified science through the development of such concepts as energy flow. ☐

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Embryological cookery book

Methods in Mammalian Reproduction. Edited by J. C. Daniel. Pp. 566. (Academic: New York, San Francisco and London, 1978.) $39.50; £25.65.

TEXTS that tell you how to do things, whether they be cookery books or manuals of bee-keeping or motor-cycle maintenance, can be valuable both for the experienced and for the novice; they also have a particular fascination for the voyeur, who has no intention of actually keeping bees or maintaining motor-cycles, but likes to identify with the contortions of those who do. Even the best of such books can be dangerous, however, if they are accepted as an alternative to visiting the master, watching him perform, and then trying the procedure yourself under his critical eye.

With this caveat, *Methods in Mammalian Reproduction* is warmly to be welcomed. Its predecessor, *Methods in Mammalian Embryology,* which appeared in 1971 with the same editor but a different publisher, must have achieved an extremely high average Citation Index rating; my own copy is constantly borrowed. The present volume has a very similar mix of 'developmental' and 'reproductive' topics to the previous one, despite its title, but the themes are now a little more sophisticated. For example, induction of ovulation has been succeeded by *in vitro* oocyte maturation, fertilisation by parthenogenesis, aggregation chimeras by injection chimeras.

Most of the book is concerned with embryos and the remarkable things that can be done to them during the pre-implantation period (oocyte maturation, experimental parthenogenesis, manipulation of ploidy, injection chimeras, immunological interference with development) or subsequently (embryo culture during and after implantation, embryo grafts to the chick, limb regeneration). Others describe techniques that can be applied more generally during development (freezing, tissue separations, one- and two-dimensional gel electrophoresis, and fluorography). Then there are chapters on particular groups of animals (marsupials; primates; embryo culture in rabbits, sheep and cows; embryo transfer in farm animals). Finally, there are a few rather heterogeneous chapters on the maternal side, dealing with pheromones, experimental delay of implantation, female genital tract secretions, intra-uterine devices in monkeys and rodents, grafting endometrium to ectopic sites, laparoscopy (not in humans) and amniocentesis (only in humans).

Some chapters are long and very detailed: gel electrophoresis; injection chimeras; amniocentesis, including cell culture and chromosome banding. Some read more as interesting accounts of rather esoteric research fields than as descriptions of widely used techniques: endometrial transplantation; immunological inhibition of development (mainly with antisera against zona pellucida); regeneration of mammalian limbs (they don't, even with electrical stimulation, but bits of brain implanted in the stump help, and bone, muscle and nerve individually do quite well).

Bibliographies are satisfactory only up to 1976, with some 1977 papers listed as being in press; the descriptions of some rapidly advancing areas, such as the use of ultrasound and amniocentesis in prenatal diagnosis, tend in consequence to be a little dated. Editing could be improved: for example, techniques for the recovery of embryos from monkey reproductive tracts are distributed among three chapters, laparascopy, IUDs, and primates; no cross-references are given. The index is inadequate.

These are minor criticisms. If you have found Joe Daniel's *MME* useful, you will want to possess *MMR* also. The initial purchase price is high, but you are likely to recoup it many times over, by increased efficiency, avoidance of costly errors, and some appreciation of what you may be letting yourself in for.

Anne McLaren

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Dioxin workshop

Dioxin: Toxicological and Chemical Aspects. Edited by F. Cattabeni, A. Cavallaro and G. Galli. Pp.222. (SP Medical and Scientific Books/Spectrum: New York and London; 1978.) Distributed by Halsted/Wiley. £14.50; $26.50.

ON 10 July, 1976 the north Italian town of Séveso was contaminated with the discharge from a chemical reactor used for the manufacture of trichlorophenol. The discharge consisted primarily of sodium trichlorophenate, but also contained an extremely toxic by-product of the reaction 2,3,7,8-tetrachlorodibenzodioxin (TCDD).

Faced with a serious pollution problem the Province of Milan and the Lombardy Regional Government sponsored a workshop in October 1976 to which scientists working on polychlorinated dibenzodioxins (PCDDs) were invited. This book records the workshop proceedings.

Research on the toxicology of the PCDDs has increased at an exponential rate since 1976 Recently published results, for example, have shown, that TCDD will cause tumours in experimental animals. As the workshop proceedings contain no discussion of the potential carcinogenicity of the dibenzodioxins and make only cursory reference to mutagenicity studies, its coverage of the toxicology of these compounds is incomplete.

That section of the book dealing with the chemistry is, nevertheless, useful. Accounts are given of methods currently in use for the analysis of environmental samples containing PCDDs by various combinations of gas liquid chromatography, mass spectrometry and mass fragmentography; and there is some discussion of the problems encountered in separating dibenzodioxins from other industrial pollutants (for example, polychlorinated biphenyls).

But perhaps of most interest will be the information on decontamination methods following TCDD pollution. Both incineration and photolysis are considered. Temperatures in excess of 800 °C are necessary for the destruction of TCDD—at lower temperatures (300–500 °C) more of the dibenzodioxin is formed from precursors of TCDD and 2,4,5-trichlorophenate.

The processes of photolysis are covered in four separate chapters. In the presence of a hydrogen donor such as methanol, ultraviolet irradiation of TCDD will selectively remove chlorine atoms from the molecule, thus rendering it less toxic. Alternative solvents/hydrogen donors are required for field conditions, and the action of two such combinations (ethyl oleate and xylene, olive oil and cyclohexanone) is described. Results reported from field trials at Séveso suggest that the olive oil/cyclohexanone method effectively promotes the degradation of TCDD, and that little of the dibenzodioxin was leached into the soil, despite heavy rain during the experiment.

Two potential decontamination methods not referred to are chlorinolysis and bacterial action. Their omission is not particularly serious; however, as bacteria are probably responsible for the detoxification of most of the TCDD in the environment these are organisms which cannot be ignored.

On the whole the book should prove useful as an introductory text for students of the dibenzodioxins.

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Large amplitude vibrations in molecules

Internal Rotation and Inversion: An Introduction to Large Amplitude Motions in Molecules. By D G Lister, J N Macdonald and N. L. Owen. Pp 246 (Academic: London and New York, 1978.) £11 50.

Large amplitude low frequency vibrations comprise a somewhat specialised topic within the subject of molecular vibration–rotation spectroscopy. However, they comprise a topic of particular interest for a number of reasons and are thus a deserving subject for a specialist text. The interest arises because these vibrations represent motions along the valleys in the molecular potential energy surface, so that they are of especial interest in the molecular dynamics of conformational changes and chemical reactions. Also, the experimental data on the energy levels of low frequency vibrations may be extensive (in contrast to the usual situation for other vibrations), and the theory by which they are related to the potential energy surface does not fall into the usual pattern of vibrational theory Thus, in principle a book on this subject might be of interest to a wide range of chemical physicists.

This book starts with an introductory chapter on the concept of large amplitude vibrations, with many examples, and diagrams of sections of potential energy surfaces. Chapter 2 deals with basic theory, chapter 3 gives a cursory account of experimental methods (microwave, infrared, Raman and NMR spectroscopy, and electron diffraction), chapter 4 reviews *ab initio* and semi-empirical potential surface calculations, and the remaining five chapters discuss particular examples of large amplitude vibration in more detail—essentially internal rotation, inversion and ring puckering vibrations, and conformational motions in macromolecules.

Although the physical ideas are well presented, the authors have attempted to write a book which is not too taxing in mathematical theory and quantum mechanics Sympathetic as I am to this ambition, the serious student has to face up to the theory, for indeed the relationship of experimental spectra to potential surface should be the unifying theme of the subject. Thus, I found the book weak in the very area where I feel it should be strong. The theory is loose where the student wants rigour; the material is badly organised; some important topics are not mentioned and others are discussed twice in different places without any apparent correlation. As examples of these defects: on p23 "transpose" should read "conjugate transpose"; equation (2.45) on p42 lacks a factor $\hbar^2/2I$, different methods of solving the Mathieu equation are discussed on p42 and p111 without any cross reference or comments to help the reader; and the effects of inversion on rotational constants are also discussed twice, on p58 and p70, again without cross reference (why is a first power term in x included on p70 and not on p58?), although one might reasonably have expected to find this discussion in chapter 7 or chapter 8. Many passages give me the impression that they will be of little help to a student who does not already know the subject, and of even less help to the student who does; as examples of this, consider the page about Van Vleck transformations (p25–26), or the four pages about gas electron diffraction (p81–84). The important topic of the symmetry of non-rigid molecules, developed in the past ten years by Longuet-Higgens, Hougen, Bunker, and others, does not seem to be mentioned anywhere in the book. Quasi-linear molecules are an important class of molecules with large-amplitude vibration that are not discussed.

The book has its good points; one of them is indeed the subject itself! But in today's sophisticated and competitive world of specialist scientific textbooks, I feel that it has not been written with the care and attention to detail that are necessary for success.

Ian M. Mills

Ian M Mills is Professor of Chemistry at the University of Reading, UK.

Thin films and the electronics industry

Thin Films: Interdiffusion and Reactions. Edited by J. M. Poate, K N. Tu and J. W Mayer. Pp.578 (Wiley: New York and Chichester, UK, 1978.) £25.

Many reasons are advanced to explain why our scientific and inventive abilities in the physical sciences are frequently not realised in industry. One good reason is the failure by scientists and industry alike to treat product design and manufacturing processes as vital technologies which must develop their own specialised sciences if inventiveness is to be fulfilled in the market. We all know, without effect, that this common neglect stems from our history when science developed rapidly and modern industry was founded, both occurring almost independently. This unsatisfactory division rooted in the scientist of independent means and the practical manufacturer has lead to the notions that the study of how one makes things and how they perform is too close to common work to be regarded as suitable activities for trained minds; and in any case such investment is wasteful Only during the last War did scientists effectively join with industry to develop expertise; but so quickly did we slip back that we now seem to have no defence against another crisis—that of market competition.

If one doubts the validity of these comments then it is only necessary to read recent views of the decision of the National Enterprise Board to enter the microprocessor field. Expert opinion on this relates to financial investment problems, preference for licencing overseas know-how, industrial need for microprocessors regardless of dependence on supply, and the novelty of the electronics and their social effects Nowhere have I found any mention of what must be done to found a satisfactory industry in the UK for the manufacture of large scale integrated electronics For example, who supplies the process plant? A new area of electronics cannot be established without the association of scientists and engineers developing production and product know-how and training skilled workers, that is, if the enterprise is to be more than a manufacturing unit with off-shore control of every aspect of its activities.

The book under review dealing with problems encountered in making and operating solid-state devices illustrates what must be done to establish such an industry It also shows how US companies are able to create the industrial science needed for the purpose at hand. There remarks are not intended to be disparaging of the work done by R & D scientists in solid-state laboratories in the UK but to point up the effects of inadequate R&D funding in an industry which once enjoyed a position next to the US in the world league.

The book's editors have experience of the subjects treated from research in their laboratories. Dr Poate, a one-time Harwell Fellow, is now with Bell Laboratories; Dr Tu is a specialist in solid-state diffusion at the IBM Thomas J Watson Research Center; and Professor Mayer is concerned with ion/atom collision effects at the California Institute of Technology. Their contributions are supplemented by others from research colleagues and members of the laboratories of Sandia and Philips (Amsterdam). These authors have related the thermal, chemical and electrical effects occurring in semiconducting devices to the materials and growth processes used in their fabrication. There are chapters on metal/metal interdiffusion, electromigration in thin films, electrode/compound substrate reactions, depth analysis and thin film deposition.

I read the chapters on atom transport and chemical reactions with interest because of their topical nature and because I was least well acquainted with their subjects. A new micrometallurgy is introduced in which the conditions for grain boundary diffusion, intermetallic and silicide formation and electromigration in a variety of film materials is treated over depth and surface dimensions of a fraction of a micrometer. As is essential in a book of this kind the text is well illustrated with micrographs.

I did not expect a detailed description of deposition techniques but a balanced and informed account of those in common use. Thus I question the wisdom of referring to ion beam deposition as a promising new technique while omitting mention of plasma chemical procedures which seem to have an important future in silicon technology. Also the reader is not informed that ion beam deposition is only possible when the self-sputtering yield of the incident ions is below one. Further, without experimental evidence it is unsatisfactory to suggest that ion bombardment may aid epitaxy; the case cited of poolycrystalline carbon is for deposition on a non-crystalline substrate. Generally, ion impact results in damage.

Sputtering is listed under collected deposition techniques as having the disadvantages of causing substrate damage and heating. Mention could have been made of sputtering apparatus with crossed electric-magnetic fields (magnetron) to deflect electrons from the substrate and reduce heating. It is an over simplification to lump together under "damage" particle and radiation effects which have different sources and intensities in sputtering plant operating from 10^{-3} to 10^{-1} torr gas pressure. The treatment of mixed component sputtering is not consistent. In the discussion of depth analysis (p149) it is said correctly that after an induction period components are released at rates proportional to their bulk contents, but on p95 it is stated that this only happens if there is no preferential sputtering to change the target surface composition. Few mixtures can have sputtering yields directly proportional to their contents but preferential sputtering raises the surface content of the less favoured component thereby aiding the latter's emission. As the emission of a component is proportional to the product of its surface content and sputtering yield, an equilibrium is reached under which the emission ratio is equal to the component ratio of the bulk material.

It is always dangerous to refer to a reference by way of another (in this case Chopra for Gillam) as on p95 when discussing the enriched Au layer formed on $AuCu_3$ alloy during Ar sputtering. The enriched layer is stable and results in component emission with the bulk contents ratio and not that of the enriched layer as stated on p95.

The above criticisms are of subsidiary material and they do not detract from the value of the main text in dealing with the behaviour of metal/semiconductor systems. As a reference work it will be of value to those who want to obtain a comprehensive picture of operational problems in solid-state electronics and also those who are entering the field to produce electronic devices. The authors and the Electrochemical Society (who supported the work) are to be commended for recognising its need and for its useful preparation.

L. Holland

L. Holland was director of R&D at Edwards High Vacuum, and is now responsible for the Unit for Plasma Materials Processing at the University of Sussex, UK.

Asiatic ungulates

Mountain Monarchs: Wild Sheep and Goats of the Himalaya. By George B. Schaller. Pp. 425. (Chicago University Press: Chicago and London, 1978.) £17.50.

This book, the latest in the generally excellent Chicago University Press series on wildlife behaviour and ecology, reports the results of three years of field work with the large mammals of the Himalayas, with emphasis on the wild sheep and goats of the region. The work will undoubtedly stand as the foremost source of information on these Asiatic ungulates for many years. One short chapter describes observations of the large carnivores of the region.

The book is generally organised into chapters dealing with broad biological topics such as distribution, physical attributes, herd dynamics and courtship behaviour. In each chapter, the array of species is treated according to the level of information available. Narrative or tabular summaries of the biological attributes of each species are generally lacking. This fragmented mode of presentation makes the work less readable than other books in the same series. The organisation of the book, together with its enormous geographical scope and the unevenness of information on various species, makes reading frustrating and confusing at times. Nevertheless, the author is to be commended for putting together in one volume the results of years of fieldwork under incredibly difficult conditions and an exhaustive review of the relevant literature, scanty though the latter is. Fortunately, the index is reasonably complete, although it cannot be depended upon to ferret out every piece of information, nor is it very helpful in dealing with the vast and confusing array of place-names.

The author concentrated his fieldwork on three species in Pakistan, Nepal and India: urial (*Ovis orientalis*), wild goat (*Capra aegagrus*) and bharal or blue sheep (*Pseudois nayaur*). The information on bharal is probably the most valuable and interesting. This little-known species possesses a complex mixture of sheep-like and goat-like behavioural and morphological characteristics. Schaller concludes (p44) that, "In general, bharal are considered aberrant goats, with sheep-like affinities". Again, however, the reader must refer to many pages of text, scattered throughout the book, to construct a cohesive picture of the biology of this species.

A concluding chapter on the evolution of the Caprinae provides a new perspective on this topic based on the author's own data on Asiatic caprids and literature review. This discussion of evolution and dispersal presents some counterpoints and alternative hypotheses to those of V. Geist (*Mountain Sheep*, Chicago University Press, 1971). Taken together, the two books provide a provocative basis for further research. However, as the author makes abundantly clear in his concluding remarks on the conservation of the Himalayan fauna, time is running out. Many species have been fragmented into small isolated populations, occupying habitats drastically altered by man. The reader is left with the clear impression that even the best system of reserves in the Himalayas will be inadequate unless broad changes in land-use practices can be instituted. In this regard, the enormity of the problems, economic, ecological and political, to be faced is evident.

Peter C. Lent

Peter C. Lent is with the US Fish and Wildlife Service, Anchorage, Alaska.

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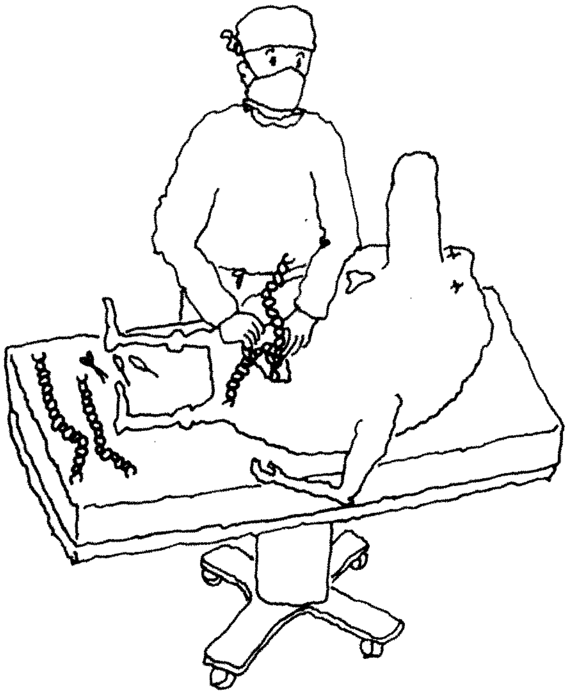

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3. BIOCHEMIST/PHYSIOLOGISTS

To carry out investigation to obtain base-line data on the physiology of reproduction of local breeds of cattle. Experience in steroid essay is required.

QUALIFICATIONS: Basic veterinary qualification is required for the first post but would be an advantage for the other two posts. In all cases applicants must have a research degree and experience in their fields of specialisation. Detailed job descriptions are available on request.

4. FOOD ENGINEER

To participate in development of food processing technologies suitable for local conditions. To be particularly responsible for food process development studies at pilot plant level and to design simple college/village scale food processing equipments. Applicants must have a university degree in Food Engineering with considerable experience in research and development work in food technology and in design of simple food processing equipment.

5. FOOD SCIENTIST/TECHNOLOGIST

To conduct investigation on post harvest physiology of local fruits and vegetables with a view to developing appropriate storage methods; to develop suitable processes for production of processed products from local fruits and vegetables and other agro-industrial by-products.

Applicants should have senior research degree in food technology with considerable experience in Research and Development work on fruits and vegetables.

Detailed job descriptions are available on request.

Salary according to qualifications and experience will be on the following scales:-

Senior Principal Professional Officer K7,812 by 204 to K8,016 by 216 to K8,232.

Principal Professional Officer K6,756 by 216 to K6,972 by 228 to K7,200 by 204 to K7,608.

Senior Professional Officer K5,424 by 288 to K5,712 by 300 to K6,012 by 312 to K6,324.

Applications giving full personal details, qualifications and experience giving names and addresses of three referees should be submitted to:-

The Secretary General
National Council for Scientific Research
P.O. Box CH 158
Chelston
LUSAKA
Zambia.

8460(A)

WRITTLE AGRICULTURAL COLLEGE

LECTURER — Grade 1A or 1B

required as soon as possible in the Science Department to teach principles of crop protection and plant physiology to H.N.D. and O.N.D. courses in commercial and amenity horticulture.

Further details and application forms obtainable from:
The Principle, Writtle Agricultural College, Chelmsford, Essex CM1 3RR.

8456(A)

OVERSEAS DEVELOPMENT

KNOW-HOW: vital to developing countries

Rice Agronomist

Tanzania

Required to work in Southern Tanzania with responsibility for executing a rice research programme. Will be stationed at Naliendele Agricultural Research Institute and will provide the main source of professional advice on rice production with priority being given to the areas selected for inclusion in the Tanzania/British Joint Programme. Will be assisted by a Field Officer and two Field Assistants provided by the Ministry of Agriculture and will directly supervise their work. Will also advise on a suitable staff training programme, including in service and overseas training.

Appointment 2 tours of 1 year. Salary (UK taxable) in range £8,800 to £10,000 p.a. plus tax-free overseas allowance in range £1,400 to £3,570 p.a. (Ref. 331 X).

The post is wholly financed by the British Government under Britain's programme of aid to the developing countries. In addition to basic salary and overseas allowances other benefits normally include paid leave, free family passages, childrens education allowances and holiday visits, free accommodation and medical attention. Applicants should be citizens of the United Kingdom.

For full details and application form please apply, quoting reference stating post concerned, and giving details of age, qualifications and experience to.

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London SW1E 5DH. 8404(A)

ODM

HELPING NATIONS HELP THEMSELVES

POLYTECHNIC OF CENTRAL LONDON

BIO-MEDICAL RESEARCH GROUP

RESEARCH FELLOW (TISSUE CULTURE)

required to establish human retinal tissue cultures for research into inherited retinal diseases The Fellow will set up the facilities required to grow cell lines and will initiate and be responsible for subsequent research projects The successful candidate will have experience of tissue culture or cell biochemistry and proven ability to undertake independent work and to provide leadership

RESEARCH ASSISTANT (RADIATION PHYSICS)

required for a project on microdosimetry of the lung to investigate the potential hazard of inhalation of fine plutonium dust Since the alpha-particles from such material travel only short distances in tissue, it is possible that the local energy deposited will give rise to effects different to those arising from homogeneous irradiation. An initial study with the rat lung lends support to this idea and the work needs extending to the human lung

RESEARCH ASSISTANT (BIOENGINEERING)

required to work with a computer based instrumentation system for measuring the forces under the normal and pathological human foot The successful candidate will be a graduate with a good honours degree in Engineering or Physics, and would be expected to register for a higher degree

RESEARCH ASSISTANT (CLINICAL AUDIOLOGY)

required to work in cooperation with a London Teaching Hospital on the measurement of human auditory function. If appropriately qualified the appointee will be registered for a higher degree.

Salary scales: Fellow £4,149 to £4,440, Assistant £3,015 to £3,189

Application forms and further details from the Establishment Officer, P.C.L., 309 Regent Street, London W1R 8AL (Tel: 01-580 2020 ext 212) Closing date. December 7, 1978. 8441(A)

UNIVERSITY OF QUEENSLAND

Australia

DEPARTMENT OF MINING AND METALLURGICAL ENGINEERING

Applications are invited for the the post of

RESEARCH ASSOCIATE

to study phase transformations in inorganic materials with special reference to reactions of gases with metal oxides Research to be carried out under the supervision of Dr P. Hayes, will involve studies of both reaction kinetics and product morphology

Applicants should hold or be about to complete a Ph.D. in Metallurgy or Physical Chemistry, experience in computing and electron microscopy desirable.

Australian Research Grants Committee post available from January 1, 1979

Salary $A13,083 to $A14,987 Assistance with travel is available for successful applicant.

Applications with curriculum vitae and the names of two referees should be sent as soon as possible to: The Staff Officer, University of Queensland, St. Lucia, Queensland 4067, AUSTRALIA. Quote Reference No 38178 8490(A)

GRADE 3 Technician vacancy in an active protein chemistry research group The work involves the running and routine maintenance of sophisticated analytical instruments. Experience with automated amino acid analysers or other similar equipment would be an advantage. Salary in the range £3,153 to £3,525 (under review) including London Weighting. Write or telephone for an application form to Departmental Superintendent, Biochemistry Department, Imperial College, London SW7 2AZ. Telephone 589 5111, Ext. 1119. 8433(A)

MASS SPECTROMETER Engineer/Technician required to join the group of Dr H. R. Morris Applicants must have experience in mass spectrometer maintenance including electronics. The person appointed will join an active group interested in instrumental development and modification. Present equipment includes MS30 and MS50 spectrometers and a DS505M data system Interests include C.I., F.D., laser and moving belt developments. The appointment will be on Technical Grade 6 scale £4,119 to £4,830 (under review) including London Weighting

Write or telephone for an application form to Departmental Superintendent, Biochemistry Department, Imperial College, London SW7 2AZ Telephone 589 5111, Ext. 1119 8432(A)

Department of Oral Anatomy
ROYAL DENTAL HOSPITAL
St George's Hospital Medical School

Cranmer Terrace, London SW17

Electron Microscopy Technician Grade 5 required salary scale £3,186 to £3,720 plus £465 London Weighting. Experience in transmission electron microscopy and associated photography essential. Knowledge of scanning EM advantageous.

Applications with names of two referees to be submitted to Dr A. F. Hayward at the above address by November 31, 1978 For further information telephone 01-672 1255, Ext. 4633. 8411(A)

BRITISH ANTARCTIC SURVEY CAMBRIDGE

SENIOR SCIENTIFIC OFFICER/ PRINCIPAL SCIENTIFIC OFFICER

The BAS has a vacancy for a GEOLOGIST to be responsible for assessing the mineral potential of the Survey's operating area Applicants must be capable of identifying areas of interest from existing rock collections and from published and unpublished data The post involves original research, both in the field and the laboratory, to examine the origins and distributions of mineralised areas This will relate directly to geochemical, petrological and geochronological studies being made at present to understand the plate-tectonic evolution of the Gondwanian and Andean belts

Applicants must be prepared to visit Antarctica to carry out field-work, excellent health, therefore, is essential **Qualifications and Experience:** Candidates should have an honours degree or equivalent in geology, plus at least 5 years relevant experience **Salaries:** Senior Scientific Officer, £5,154 to £6,898 per annum, Principal Scientific Officer: £6,609 to £8,461 per annum Starting salary may be above the minimum, according to qualifications and experience.

The staff of the Council are not Civil Servants, but their pay and conditions of service are similar to those of scientists in the Civil Service. There is a non-contributory superannuation scheme

For further details and an application form (please quote ref BAS 105) apply to the Establishment Officer, British Antarctic Survey, Madingley Road, Cambridge CB3 0ET.

Closing date: December 11, 1978

THE NATURAL ENVIRONMENT RESEARCH COUNCIL

8495(A)

THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY

Nairobi, Kenya

Applications are invited from suitably qualified persons for the following positions:

1. RESEARCH SCIENTIST OR POST-DOCTORAL RESEARCH FELLOW (TSETSE POPULATION DIVERSITY PROGRAMME)

REF: SCI/78/24

Qualifications:

Ph.D. with 5 or more years post-doctoral experience in biological sciences and a demonstrable experience in ecological or eco-genetic research with anthropods. High quality research for Ph.D. and evidence for excellence since doctorate will be a distinct advantage.

Responsibilities:

The successful candidate will carry out research in the field of tsetse population dynamics.

2. RESEARCH TRAINING FELLOWSHIPS IN INSECT ECOLOGY (7 POSITIONS)

REF: SCI/78/25

Qualifications:

Young African Scientists, holding Ph.D. or M.Sc. degrees in entomology or zoology, with bias towards ecology. High quality research capability should be proven as evidenced by thesis, dissertation, or any other relevant research experience. Scientists completing thesis work from recognised universities are also legible. The positions will be available in 1979.

Responsibilities:

The successful candidates will undertake research in ecology of livestock ticks, tsetse, medical vectors or tropical diseases (especially mosquitoes and biting flies), stem and pod borers, African armyworm, harvester termites, and sorghum shootfly, collaborating with ICIPE research scientists who are mainly working in eco-physiological research.

Research training will be carried out mainly at the ICIPE Field Stations located in the coastal, semi-arid savannah, and lake-shore regions of Kenya. Further training in advanced ecological techniques may be implemented in Kenya and abroad as the individual cases may demand. Applicants should indicate their preference in research.

3. CONTROLLER FOR INSECTARY SERVICES—

REF: SCI/78/26

Qualifications:

Candidates should be in possession of a Ph.D. or M.Sc. degrees, preferably in insect or animal pathology, but possibly also in zoology, biology, or physiology. They should have strong technical training and a solid scientific or research background at a senior level. They should necessarily be interested in insect breeding problems and techniques.

Responsibilities:

The successful candidate will be required to (a) plan the insect and animal breeding unit; (b) carry out experiments needed to establish routines for the breeding of each of the target insect species and required laboratory animals; (c) supervise the operations of the insect and animal breeding unit; and (d) train staff working under him. It is expected also that he will monitor the health of insects under his care. It is hoped that the successful candidate will be able to commence work immediately.

GENERAL INFORMATION FOR ALL POSITIONS:

Salary:

Negotiable and include attractive benefits.

Applications giving detailed information or general education, professional qualifications, experience, marital status, age, present terms of employment (including salary), names and addresses of four referees (including one personal), and photostat copies of relevant certificates and diplomas should be addressed to:

Administrative Manager,
I.C.I.P.E.
P.O. BOX 30772,
NAIROBI.

to reach him not later than 20th December 1978.

8412(A)

unilever research

Pathologists

Due to expansion our Environmental Safety Division requires–

(1) One pathologist with experience.
(2) Two veterinary graduates interested in becoming pathologists

Successful applicants for these posts would be encouraged to commence or complete preparation for the membership examinations of the Royal College of Pathologists.

Our Environmental Safety Division employs almost 200 people whose prime job is to establish the toxicological safety of Unilever Products Pathologists do autopsies on small animals, make histopathological examinations and prepare reports. Staff are encouraged to undertake more specialised research in the field of toxicology/pathology

The division forms part of the Unilever Research, Colworth Laboratory which occupies a 1300 acre estate, ten miles North of Bedford.

Successful candidates will be eligible for BUPA and assistance with removal expenses.

Please write quoting Ref CH-200M and giving details of career history to:

Miss J. Kenny,
Personnel Officer,
Unilever Research,
Colworth Laboratory,
SHARNBROOK,
Bedford MK44 1LQ.

8429(A)

PHARMACOLOGISTS

Weddel Pharmaceuticals Ltd., a subsidiary of the Union International Co. Ltd., wish to recruit two PHARMACOLOGISTS for the Pharmacology Department at the Groups' Research Centre at St. Albans, Herts.

The work involves biological studies on naturally-occurring inhibitors of mitosis and expertise in oncology, immunology or haematology would be an advantage.

Applications are invited from Biological Scientists with a postgraduate degree and the ability to make a significant contribution to the output of an active, expanding department.

Please write, giving full personal and career details including present salary, to The Staff Manager (AD 6071) Weddel Pharmaceuticals Ltd., 14 West Smithfield, London EC1A 9JN. 8463(A)

AN FORAS TALUNTAIS

GRADUATE IN AGRICULTURAL SCIENCE OR VETERINARY SCIENCE

Required for research in cattle breeding at Belclare Research Station, Tuam, Co. Galway.

The appointee will be considered for a permanent post after an initial two years employment. Commencing salary will depend on qualifications and experience.

ESSENTIAL *either* an honours University Degree or equivalent in Agricultural Science *or* a degree in Veterinary Science or equivalent.

DESIRABLE appropriate post-graduate qualification and—or research experience.

Application forms and further particulars from Personnel Department, An Foras Taluntais Headquarters, 19 Sandymount Avenue, Dublin 4 (Phone (01) 688188). Latest date for receipt of completed entries is Thursday, November 30, 1978.

8402(A)

UNIVERSITY OF ESSEX

DEPARTMENT OF CHEMISTRY

CHAIR IN BIOLOGICAL CHEMISTRY

Applications are invited for a newly established Chair of Biological Chemistry in the Department of Chemistry for appointment from October, 1979 Preference will be given to applicants in one of the following areas enzymology, bio-inorganic chemistry or in the application of physico-chemical methods to biological systems

Applications (12 copies, quoting AG/56/N), including a curriculum vitae and the names and addresses of three referees, should reach the Registrar, University of Essex, Wivenhoe Park, Colchester CO4 3SQ, from whom further particulars may be obtained, by December 15, 1978

8427(A)

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF ANATOMY AND EMBRYOLOGY

RESEARCH ASSISTANT

(Grade IB)

required by Professor G Burnstock to participate in research on the Autonomic Nervous System Experience in pharmacology, electron-microscopy immunohistochemistry or neurochemistry an advantage Appoint- for 1 year in the first instance Applications including full c v and names of two referees to Assistant Secretary (Personnel), (N) University College London, Gower Street, WC1E 6BT 8474(A)

UNIVERSITY OF LONDON

INSTITUTE OF NEUROLOGY

POSTDOCTORAL NEUROCHEMIST/ BIOCHEMIST

Required to join a team studying the neurochemistry of memory disorders 2-year contract, salary according to age and experience

Applications stating age, qualifications, experience and career goals should be sent to Dr David M Bowen, Department of Neurochemistry, 33 John's Mews, London WC1 2NS

8472(A)

UNIVERSITY OF EDINBURGH

DEPARTMENT OF FOREST
AND NATURAL RESOURC

Applications are invited for
post of Lecturer in Forest Mana
ment.

Applicants should have a Univ
sity degree in forestry and pract
experience of Forest Managem
Additional experience in a cogn
field, such as mensurat
statistics, economics, resou
appraisal or wildlife managem
is desirable. The successful can
date will become a member o
Department covering a wide ra
of topics in ecological science
the management of renew
natural resources and will
expected to cooperate in teach
in integrated ecology courses
well as in Forest Management:
will also be expected to pursue
promote research on relev
topics.

The successful candidate will
expected to take up the post
on October 1, 1979.

Salary scale: £3,883 to £7,
per annum.

Applications (three cop
together with the names of t
referees should be lodged not la
than January 23, 1979 with
Secretary to the University,
College, South Bridge, Edinbu
EH8 9YL, from whom furt
particulars may be obtain
Please quote reference 1079.

8408(A

UNIVERSITY OF EDINBURGH

DEPARTMENT OF GEOPHYSICS

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for
post of Postdoctoral Resea
Fellow to work on the South
Uplands Seismic Refraction Prof
The project is collaborative w
the Global Seismology Unit of
Institute of Geological Sciences
is designed to investigate tempor
radio-linked extensions of
permanent local seismic netwo
LOWNET

The appointment is for th
years with a salary of £4,382
£4,882 p a and immediate av
ability Requests for furt
information and applicatio
including a full curriculum vi
and two referees, should be m
to Dr R G Hipkin, Departm
of Geophysics, J.C M.B , Mayfi
Road, Edinburgh EH9 3JZ Ple
quote Reference 5063

8423(A

THE UNIVERSITY OF LANCASTER

DEPARTMENT OF PHYSICS

RESEARCH ASSOCIAT

Applications are invited for
post of postdoctoral RESEAR
ASSOCIATE in the Low Tempe
ture Physics group The post
financed by S R C for THR
YEARS from January 1, 1979,
the R A 1A scale £3,883 to £6,5
under review) The resear
associate will work with
Pickett and Guenault on exp
ments below 1mK on superfl
helium-3 A nuclear demagnet
tion refrigerator constructed
this research programme is n
nearing completion

Further details may be obtai
(quoting reference L91/E) from
Establishments Officer, Univer
House, Lancaster LA1 4YW
whom applications (5 cop
naming two referees, should be
not later than November 30, 19

8450(A

UNIVERSITY OF LEUVEN BELGIUM

RESEARCH ASSOCIATE in VIROLOGY

Applications are invited for the post of Research Associate, tenable until July 1980 with extensions up to 4 years possible, to assist in research mainly on basic aspects of viral hepatitis, but also on other subjects of medical virology.

Research experience in virology is necessary, and proficiency in biochemistry, immunology or electron microscopy would be important assets. The candidate should have a Ph.D. or M.D. with thesis, or equivalent credentials. A working knowledge of English is desired.

Salary is in the range of 19,000 to 33,000 US dollars per annum, depending on age and qualifications. It is indexed to the cost of living, and social provisions including health insurance are as for Belgian nationals.

The position is open immediately. Applicants are invited to submit now their curriculum vitae, list of publications, addresses of two referees, and the date at which they are free to: Professor J. Desmyter, Rega Institute for Medical Research, Minderbroederstraat 10, B-3000, Leuven, Belgium.

8394(A)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF GENETICS

TECHNICIAN GRADE 5

A vacancy exists for a laboratory technician to join a small group investigating DNA repair and recombination mechanisms in the bacterium **Escherichia coli.** The person appointed will be expected to assist with research work and collate data for analysis, in addition to carrying out general laboratory maintenance.

Minimum qualifications H.N.C. or equivalent although a degree in an appropriate discipline would be an advantage. Preference will be given to candidates with previous experience in microbiology, genetics or biochemistry.

The position is funded by the S.R.C. and will be for a period of one year in the first instance.

Salary within the range of £3,186 to £3,720 per annum.

Applications in writing, stating age, experience, qualifications and the names of two referees should be sent to the Establishment Officer, University of Nottingham, University Park, Nottingham NG7 2RD. Ref. No. GN/10/78. 8437(A)

CHEMIST/BIOCHEMIST

GENETICS RESEARCH UNIT

ROYAL CHILDREN'S HOSPITAL RESEARCH FOUNDATION

Flemington Road, Parkville,
Melbourne, Victoria 3052, Australia.

Position available in group studying inborn errors of metabolism for a Postdoctoral Scientist with experience in organic chemistry, biochemistry or neurochemistry and a particular interest in human biochemical genetics. Opportunity to develop own field of interest and to contribute to existing projects. Particular needs exist for development of new methods of detecting and identifying metabolites in body fluids and tissues. Expertise in intermediary metabolism, enzymology or protein chemistry also valuable.

Salary on Senior Research Officers scale of N.H. and M.R.C.—dollars A15,179 to A19,940 per annum.

Applications (in writing) or enquiries to Professor David Danks. 8415(A)

M.I.T.

Postdoctoral and Predoctoral Research Positions

Several positions are available for research in genetic toxicology with emphasis on mechanisms of chemical mutation of human cells. Strong background in chemistry is desired of predoctoral researchers, and molecular biology of postdoctoral researchers. Please send resumes to Professor William G. Thilly, Room E18-666, M.I.T., Cambridge, MA 02139. M.I.T. s an equal opportunity employer.

8392(A)

SIMON FRASER UNIVERSITY

BRITISH COLUMBIA, CANADA

DEPARTMENT OF BIOLOGICAL SCIENCES

ENTOMOLOGIST

The Department of Biological Sciences invites applications for a tenure track position in entomology. The successful applicant will be expected to develop a strong research programme and to participate in the teaching programme at both the undergraduate and graduate level including courses in the Master of Pest Management Programme. Candidate must have a Ph.D. degree in entomology or related subjects; preference may be given to individuals with teaching and research experience in agricultural pest management. The position is available, subject to budgetary constraints, from September 1, 1979. The initial appointment is for a period of two years, renewable. Salary will depend on experience; the base salary for the Assistant Professor rank currently is $18,604 per annum.

Applications, including an up-to-date curriculum vitae, reprints of published research, and the names of three referees should be sent to: Dr M. Mackauer, Chairman, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada, V5A 1S6. Deadline for receipt of applications is January 31, 1979 or when position filled. 8390(A)

UNIVERSITY OF READING

LECTURESHIP IN FOOD QUALITY CONTROL

Applications are invited for a Lecturership in food quality control tenable at the National College of Food Technology, Weybridge, which is part of the University. Candidates should hold appropriate qualifications and have knowledge and experience of quality control systems and/or techniques of food analysis. The successful candidate will be expected to develop an experimental research programme in a relevant field of study, and contribute to the teaching at undergraduate and postgraduate levels.

The person appointed should take up duties as soon as possible.

Further information may be obtained from the Registrar (Room 214, Whiteknights House), The University, Whiteknights, Reading RG6 2AH, by whom applications should be received not later than December 16, 1978.

8443(A)

PROFESSOR OF MOLECULAR BIOLOGY

The Department of Molecular Biology of Vanderbilt University seeks to fill a senior capacity position at the level of Professor or Associate Professor. Candidates should have a noteworthy research programme in the chemical or physical aspects of molecular biology. Ample laboratory space and financial support to facilities relocation of the research programme are available. The teaching responsibilities are modest, permitting emphasis on research and research training. Salary negotiable. Informal inquiries are welcome. Alternatively, please send curriculum vitae, biblography, a description of research and teaching interests, and names of three references to: Dr Oscar Touster, Department of Molecular Biology (Box 1820, Station B.), Vanderbilt University, Nashville, Tennesse 37235. An Affirmative Action/Equal Opportunity Employer. 8393(A)

CHELSEA COLLEGE

University of London

TECHNICIAN 2B

required for small but active Human Biology Department in the Basic Medical Sciences Group. Duties will include assistance in both teaching and research areas, tissue culture experience an advantage. Salary within the range £2,993 to £3,345 per annum inclusive of London Allowance.

Application forms from Personnel Office, Chelsea College, University of London, Friese Greene House, Chelsea Manor Street, London SW3, to be returned by November 30, 1978.

8489(A)

Organic Chemist

Pesticide Research

The Plant Protection Division of ICI wishes to recruit two PhD Organic Chemists to lead small teams concerned with the discovery of novel compounds with pesticidal or plant growth regulating activity. We seek imaginative scientists of proven research ability, able to conceive and carry out new ideas for the design of biologically active molecules. The interests of the successful candidates will extend beyond the bounds of organic chemistry to include an appreciation of structure-activity relationships, biochemistry and biology. The job demands the ability to work constructively as a member of a multi-disciplinary project team.

The position will appeal to candidates who have experience in the field of pesticide or medicinal chemistry, either in industry or in a university post doctoral position.

Jealott's Hill is the centre for pesticide research and development work carried out by the Plant Protection Division of ICI. The Research Station is situated in pleasant rural surroundings between Bracknell and Maidenhead in Berkshire.

We offer competitive salaries and the Company has a Profit-Sharing Scheme and Contributory Pension Scheme. Assistance with removal expenses and house purchase may also be available.

If you wish to apply please write or telephone for an application form to:

Mr. D. Spence, Personnel Officer, ICI Plant Protection
Division, Jealott's Hill Research Station,
BRACKNELL, Berkshire RG12 6EY
BRACKNELL 24701 Ext 3350

ICI

Plant Protection Division

8457(A)

PROFESSOR OF NATURAL SCIENCES

The Institute for Advanced Study invites applications and nominations for the post of professor of natural sciences. This is a tenured position. Candidates should have distinguished records of achievement in research and must demonstrate the capability of further significant research contributions. The ability to stimulate and assist visiting postdoctoral members is an important qualification for the position. Theoretical research is preferred; no laboratory facilities are available. Professors are required to be in residence two terms each year. Women and members of minority groups are encouraged to apply.

Please address all inquiries and applications to Dr Harry Woolf, Director, The Institute for Advanced Study, Princeton, New Jersey 08540. Applications should include a vita and a list of publications. 8461(A)

KING'S HEALTH DISTRICT (TEACHING)

TRAINEE TECHNICIAN

to work in the Pharmacy Quality Control Laboratory to assist with the analysis of medicinal products, using advanced instrumentation. Applicants should possess one or two 'A' levels or five 'O' levels (including Chemistry). Possibility of day release to study for H.N.C. or O.N.C. SCIENCE GRADUATES WILL BE CONSIDERED.

Starting salary, according to age and qualifications, within range: £2,256 (at age 16) to £3,264 p.a. inclusive.

Further information from Miss Hodges on ext. 2472. Application form and job description available from the District Personnel Department, King's College Hospital, Denmark Hill, London SE5 9RS. Tel: 01-274 6222 ext. 2254.

Closing date: December 1, 1978.

8442(A)

UNIVERSITY OF SINGAPORE

CHEMICAL ENGINEERING

PROFESSORSHIP

Applications are invited for appointment as Professor in the Department of Chemical Engineering. The successful candidate should have considerable teaching, industrial and administrative experience. Gross monthly emoluments in the range from S$3,745 to S$5,775 approx., the initial amount depending on the candidate's qualifications and experience In addition, the University pays a 13th month annual allowance of one month's salary in December of each year. Leave, subsidised housing, medical and provident fund benefits are available. Candidates should write to: *The Registrar, University of Singapore, Kent Ridge, Singapore 5,* giving curriculum vitae (bio-data), with full personal particulars and also the names and addresses of three referees. 8395(A)

BHP

DIRECTOR

MELBOURNE RESEARCH LABORATORIES

AUSTRALIA

The Broken Hill Proprietary Co. Limited (BHP) is the largest Company in Australia, with annual sales of $A2,374 million and employing some 59,000 people in a variety of activities covering steel, minerals, oil and gas and manufacturing. These activities, which range through exploration, mining, extraction, transportation and fabrication, are supported by extensive research and development programmes.

Following the promotion of Dr. W. J. McG. Tegart to an executive position at Head Office, a new Director is being sought for BHP Melbourne Research Laboratories which are one of two corporate laboratories for the BHP Group. The Director will be responsible for the total research programme in materials research and development, metal forming, mill technology, welding, product development, transportation, energy, and engineering. While the major effort is directed to the BHP Group, substantial contract work is being carried out for external companies and for Government bodies.

The Laboratories are situated on 10 hectares at Clayton, a suburb of Melbourne and were opened in 1968. They currently employ 140 people, of whom 65 are professionals. The Laboratories are well equipped by world standards and are essentially self-contained with respect to services. Their annual budget is of the order of $A3.5 million.

Applicants should have research experience and a proven record in management. Salary will be in the range $A30,000-40,000 p.a. depending upon experience and qualifications, and a Company car is provided. The Company operates a generous superannuation scheme and reasonable relocation and removal expenses will be paid. Subsidized accommodation will be provided for up to six months on arrival in Australia.

Applications containing full personal particulars, including age, qualifications, experience, publications, telephone number and name of referees should be marked "Confidential" and addressed to:

Dr. R. G. Ward,
General Manager Research and
New Technology,
The Broken Hill Proprietary Co. Ltd.,
Melbourne. Victoria. Australia. 3001

Applications should reach the above address by 15th January, 1979

BHP 454/OS

8386(A)

Anglian Water Authority

ASSISTANT TEAM LEADER

(Mathematics)

Post Ref: H6R003 Grade 6

£5,133–£5,493 p.a.

Applications are invited for the above post currently based at the Great Ouse River Divisional offices, Cambridge. The successful applicant should preferably be a graduate in mathematics with experience in the water quality modelling of estuaries, coastal waters and reservoirs, and the organisation and collection of associated data. In addition to his individual projects, the successful applicant will also be expected to assist other team members in all aspects of their varied chemical and biological investigations, including computer programming, data processing and statistics.
The post carries a Regular User Car Allowance and N.J.C. conditions of service, including a contributary pension scheme, apply.
Please wrote, or telephone 0223 61561 ext. 259 for an application form (returnable by Monday 11th December, 1978) to Mr. M. J. Pearson, Regional Research Team Leader:—

GREAT OUSE RIVER DIVISION,
Great Ouse House, Clarendon Road,
Cambridge, CB2 2BL 8486(A)

THE UNIVERSITY OF HULL SENIOR LECTURER/ LECTURER IN APPLIED PHYSICS

Applications are invited for the post of Senior Lecturer/Lecturer in the Department of Applied Physics for work on satellite laser ranging, tenable from a date to be arranged.

Salary scale: £3,883 to £7,754 per annum (under review) plus superannuation benefits.

Applications (6 copies) giving details of age, qualifications and experience, together with the names of three referees, should be sent by December 4, 1978 to the Registrar, The University of Hull, Hull HU6 7RX from whom further particulars may be obtained. 8453(A)

NORTHWESTERN UNIVERSITY

DEPARTMENT OF GEOLOGICAL SCIENCES

A new, regular assistant professorship is available September, 1979. Al applicants with strong quantitativ backgrounds in the geological science should forward, as soon as possible their curriculum vitae, list of publications, and a statement of researc interests and goals to:

E. H. Timothy Whitten, Chairman
Department of Geological Sciences
Northwestern University,
Evanston, Illinois 60201, U.S.A.

Northwestern University is a equal-opportunity / affirmative-actio employer. 8417(A)

Ministry of Agriculture, Fisheries and Food, Pest Infestation Control Laboratory, Slough

Acarologist

■ **Join small team ■ Study the biology and systematics of storage mites ■ Examine the relationship between population increases and environmental conditions ■ Investigate effect of nutrition and feeding habits of mites on the development of infestation in stored cereals.**

☐ 1st/2nd hons degree or equivalent in an appropriate biological subject ☐ At least 2 years experience in acarology required ☐ Appointment as Higher Scientific Officer (£4375–£5725) ☐ Ref: SB/39/AF.

■ Application forms (for return by 7 December 1978) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke (0256) 68551 (answering service if office closed).

Science Group
CIVIL SERVICE

8440(A)

PLANT BREEDING INSTITUTE

APPOINTMENT

OF

DIRECTOR

Applications are invited for the post of Director of the Plant Breeding Institute, Cambridge, which was recently vacated by the Director, Dr R. Riley FRS, on his appointment as Secretary to the Agricultural Research Council in October 1978. The Institute, which is grant-aided by the Agricultural Research Council, has its own Governing Body and is engaged in research in plant breeding and relevant sciences directed towards the improvement of agricultural crops. Approved varieties bred at the Institute are marketed through the National Seed Development Organisation.

Applicants should have high scientific qualifications and research experience relevant to the Institute's work. The post is graded Chief Scientific Officer. Salary is £13,047 per annum and it is the Government's intention that the salary for this grade will be £13,908 per annum from April 1979 and £14,770 per annum from April 1980.

Applications, including the names of three referees, should be received by 31 January 1979 and should be addressed to the Secretary, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, from whom further particulars may be obtained.

8447(A)

LECTURER/ASST. PROFESSOR AT THE UNIVERSITY OF TROMSOE, NORWAY

Applications are invited for a position as lecturer/asst. professor in cytology/plant physiology at the Institute of Biology and Geology. University of Tromsoe in Tromsoe, Norway. The succsessful candidate is expected to teach undergraduate and graduate courses to students in biological sciences. Teaching responsibilities also include a graduate techniques course in electron microscopy and cytology. Instruction can initially be done in English, if necessary.

The position also provides an opportunity to conduct cytological/cytochemical research. Persons with research interests in disease resistance of plants and the biochemical basis for pathogenicity in microorganisms are especially encouraged to apply. The University of Tromsoe is unique in being the most northerly situated university of the world. Its Institute of Biology and Geology has completely new facilities for conducting research including fully equipped laboratories for physiological and biochemical analyses, growth chambers and a phythotron. Computer terminals and exellent library services are also available. The electron microscope laboratory is fully equipped for all types of scanning and transmission electron microspopy.

The position is available immediately. Salary from 91439 Nkr. to 114392 Nkr. depending on qualifications.

Application deadline: Des. 20th. 1978.

For further information contact: **Sylvia Labugt, Institute of Biology and Geology, University of Tromsoe, P. O. Box 790, N-9001 TROMSOE, tph.no. (083) 70011.**

8413(A)

Ministry of Agriculture, Fisheries and Food, Plant Pathology Laboratory, Harpenden

Agriculturist/ Horticulturist/ Biologist

■ Make field surveys and visit farmers to determine the extent and methods of use of pesticides ■ Process data by computer.
□ Degree, HNC or equivalent in agricultural, horticultural or biological subject □ Experience in computing advantageous □ Possession of current driving licence essential □ Age normally under 27 □ Appointment as Scientific Officer (£2840–£4410) □ Ref: SB/18/AG.

■ Application forms (to be returned by 13 November 1978) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke (0256) 68551 (answering service if office closed).

Safety in the Food & Cosmetic Industries

Information Scientist

. . . to work on problems associated with the safety in use of cosmetics, food-contact materials and medical devices. The work includes scanning literature for relevant articles for reference files, carrying out literature surveys on various chemicals associated with cosmetics and food-contact materials, preparing reports and papers for consideration by expert advisory committees, collating and evaluating data, and consulting with representatives of the cosmetic and food industries.

Candidates, normally aged under 30, should have a degree or equivalent qualification in a relevant discipline and several years postgraduate experience. A knowledge of toxicology would be an advantage.

Appointment as Higher Scientific Officer (£4560–£5910). Starting salary according to qualifications and experience. Non-contributory pension scheme. Promotion prospects.

For further details and an application form (to be returned by 9 November 1978) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). *Please quote ref: SB/3/KE.*

Department of Health & Social Security

8478(A)

SENIOR FACULTY POSITION DEPARTMENT OF ASTRONOMY AND STEWARD OBSERVATORY, UNIVERSITY OF ARIZONA

The University of Arizona expects to make a senior faculty appointment (Associate or Full Professor) in the Department of Astronomy and Steward Observatory. The department has an interest in increasing its involvement in theoretical astrophysics and in space related, optical and infrared research. Applicants with distinguished research records in any areas of astronomy and astrophysics will, however, be considered seriously. Research facilities available include the 4.5 meter Multi-Mirror Telescope, 2.3 and 1.5 meter telescopes, and the University's CDC Cyber 175 and Dec 10 computers. Interested scientists are requested to send a vitae and biblography by February 15, 1979 to either Dr Rodger I. Thompson, Chairman: Search Committee, or Dr Peter A. Strittmatter, Director, Steward Observatory, University of Arizona, Tucson, AZ 85721. The University of Arizona is an Equal Opportunity/Affirmative Action Title IX/Section 504 Employer.

8420(A)

Experienced Toxicologists

We are looking for two established toxicologists to join our Toxicology and Drug Safety Unit

They will work in our Animal Studies Section and be expected to take an active role in the design, conduct and evaluation of experiments forming part of the safety assessment of a wide range of potential human and veterinary medicines The abilities to guide the work of less experienced staff and to communicate information are essential. Applicants of either sex are likely to have a good Honours degree, but in any event must possess at least three years' directly relevant experience. For one of these openings, some expertise in the area of Reproductive Toxicology would be an advantage but is not essential.

Remuneration will reflect the experience and qualifications of the successful applicants and re-location expenses will be paid in approved circumstances

If you wish to apply, please write to:-
John Atkinson, Site Personnel Manager,
Beecham Pharmaceuticals – Research Division, Medicinal Research Centre,
The Pinnacles, Harlow, Essex CM19 5AD

or telephone our answering service on Harlow (0279) 419373 for an application form, quoting our reference 54/N

Beecham Pharmaceuticals

8435(A)

CNS Pharmacologist

Roussel Laboratories Limited is part of an international group engaged in research, development, manufacture and marketing of a wide range of pharmaceutical and allied products.

We wish to recruit a graduate pharmacologist to fill a key role in a project team investigating and developing novel compounds with activity on the central nervous system Ideally, we are looking for one or two years' post-graduate experience in this area although we will consider candidates who have recently obtained a relevant degree or equivalent qualification

The Research Division is based at our modern manufacturing laboratories near Swindon Conditions of service are excellent, including generous assistance with relocation to this attractive part of Wiltshire

Please write or telephone for an application form to.
Rosemary Curtis, Roussel Laboratories Limited, Kingfisher Drive, Covingham, Swindon, Wilts SN3 5BZ
Tel. Swindon (0793) 24411.

ROUSSEL

8436(A)

UNIVERSITY OF SOKOTO— NIGERIA

Applications are invited for the following posts in the DEPARTMENT OF GEOGRAPHY:

PROFESSOR

Must have considerable teaching experience at university level plus administration and research experience Should have academic interest in Africa or the tropical world. Will be expected to organise, develop teaching and research in the Department and provide leadership.

SENIOR LECTURER/ LECTURER

Preference will be given to candidates who have specialised in Physical Geography and are qualified to teach Geomorphology or climatology. Must have Ph D and between 3 to 5 years of teaching and research at University level.

Salary scales Professor, N11,268 to N12,420 per annum (£8,942 to £9,857 p.a.), Senior Lecturer, N7,764 to N8,724 p a (£6,161 to £6,923 p a sterling); Lecturer N5,460 to N6,984 p.a (£4,333 to £5,542 p a sterling) (£1 sterling=N1 26.) There may be salary supplementation for Professors and Senior Lecturers in range £2,160 to £2,916 p a. (sterling) for married appointees or £NIL to £750 p a (sterling) for single appointees (reviewed annually and normally free of all tax) plus provision of children's education allowances and holiday visit passages. There will not be salary supplementation and associated benefits for appointees to Lecturer level Family passages, car allowance, housing allowance/partly furnished accommodation Fringe benefits include pension scheme or contract addition of salary (partly taxable)

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent to Registrar, University of Sokoto, PMB 2346, Sokoto, Nigeria, by December 19, 1978.

Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 8493(A)

THE UNIVERSITY OF LEEDS
DEPARTMENT OF EARTH SCIENCES

Applications are invited for the post of RESEARCH ASSISTANT or RESEARCH FELLOW for research in CHEMICAL OCEANOGRAPHY The successful candidate will work on a NERC funded programme concerned with the geochemical cycling of lanthanides and transition metals in marine sediments. The work is part of a collaborative project on oceanic processes involving the University of Leeds, the Open University, the University of Southampton and the Institute of Oceanographic Sciences, Wormley. It involves sample collection aboard RRS Discovery, and analyses using radioisotope-dilution and isotope-dilution techniques. Applicants should be graduates with experience in chemical techniques and must be willing to take part in research cruises involving absences abroad, and in visits to other research laboratories. For appointment at the Research Fellow level a Ph D. degree is required. The appointment will be for a fixed period of three years.

Salary at an appropriate point on either the IB Scale (£3,384 to £4,882) or the IA Scale (£3,883 to £6,555) for Research and Analogous Staff Both salary scales are currently under review.

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 49/9/D. Closing date for applications December 10, 1978.

8479(A)

QUEEN ELIZABETH COLLEGE
Kensington
(University of London)
POSTGRADUATE RESEARCH ASSISTANT
Human Gene Localisation

Applications are invited from graduates in Biochemistry, Molecular Biology or related subjects to assist in a project, supported by the Medical Research Council, on in situ hybridisation for human gene localisation. The appointment will be for 2 years, 9 months, in the Department of Biochemistry. Salary up to £4,408 p.a. (Range 1A) plus London allowance of £450 p.a.

Applications to Professor D Robinson, Biochemistry Department, Queen Elizabeth College, Campden Hill Road, London W8 7AH

8473(A)

CELL BIOLOGIST

The Department of Biology at the University of California at Riverside has a faculty position at the level of Assistant Professor available September 1, 1979 Primary teaching responsibilities will be in the area of cell biology both at the undergraduate and graduate levels. Candidates must have postdoctoral experience and show evidence of ability to initiate and sustain a productive high quality research program. The department is especially interested in candidates with research experience in biochemical, genetic and/or electron microscopical approaches to the study of cell ultrastructure, morphogenesis, cell surfaces, and cell interactions

Applicants should provide a curriculum vitae, reprints and a brief description of research and teaching interests Additionally, at least three letters of support should be sent. The University of California is an equal opportunity and affirmative action employer Applications are invited from all qualified individuals, especially women and members of minority groups.

Send applications to W L Belser, Chairman of the Search Committee, Department of Biology, University of California, Riverside, Calif. 92521

8476(A)

Ayrshire and Arran
Health Board
North Ayrshire and
Arran District

BALLOCHMYLE HOSPITAL
SENIOR CYTOGENETICIST

Senior Cytogeneticist (Science Graduate) required for Ayrshire Area Laboratory to work in a developing diagnostic service for the detection of human chromosome aberrations.

Appropriate qualifications and experience in cytogenetics and cell culture are essential. The post will initially be based at Ballochmyle Hospital but will move to the new North Ayrshire District General Hospital in 1979.

Further details may be obtained from the Pathology Department, Ballochmyle Hospital (Catrine 281, ext. 23).

Applications with full details of qualifications and experience and names of two referees, to the North Ayrshire District Medical Officer, 1 Hill Street, Kilmarnock. 8425(A)

The Division of Clinical Nutrition,

THE HOSPITAL FOR SICK CHILDREN

Toronto, Ontario

is seeking applicants for a position as Research Associate (full time staff member). The applicant should have received his/her doctorate in Nutritional Biochemistry or Animal Nutrition, and shoult have had one or two years postdoctoral experience. The applicant will be expected to conduct independent research in Nutrition; both in human and in animal models. The position will also entail participating in the teaching load of the Division. Applicants are requested to correspond with Dr P. B. Pencharz, Head, Division of Clinical Nutrition, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8. 8410(A)

POSITIONS AVAILABLE THREE FACULTY POSITIONS

Two Associate or Full Professors in Organic Chemistry or related chemical research area. One tenure track Assistant Professor in Analytical Chemistry. Applicants for the tenured Associate or Full Professorships should have an established research program of high quality and be experieinced teachers at the undergraduate and graduate level. Applicants for all positions must be committed to excellence in research and teaching. A complete curriculum vitae, which includes a documented summary of past accomplishments and future research plans, should be submitted to Dr Gordon Harris, Chairman of the Search Committee, Chemistry Department, State University of New York at Buffalo, Acheson Hall, Buffalo, New York 14214. Qualified female and minority applicants are encouraged to apply. Deadline for applications is January 31, 1979.

THE STATE UNIVERSITY OF NEW YORK AT BUFFALO IS AN EQUAL OPPORTUNITY/AFFIRMATIVE ACTION EMPLOYER.

8414(A)

RICHARD DIMBLEBY
DEPARTMENT OF CANCER RESEARCH
ST THOMAS'S HOSPITAL MEDICAL SCHOOL
RESEARCH TECHNICIAN

required for post vacant from January , 1979. Particular duties concern the culture of mammalian cells derived rom human and animal tissues and esearch into the response of such cells o radiotherapy and cancer chemoherapeutic drugs. Previous experience n a cell culture laboratory is desirable but applicants with general laboratory raining can be considered. Qualificaions should include M.I.S.T. or O.N.C. or equivalent. Please write giving telephone No., curriculum vitae and name and address of two referees o Professor A. H. W. Nias, St. Thomas's Hospital Medical School, London SE1 7EH. 8477(A)

QUEENSLAND INSTITUTE OF TECHNOLOGY

BRISBANE — AUSTRALIA

SENIOR LECTURER IN PHYSIOLOGY

Applications are invited from medical or science graduates holding suitable higher qualifications. The appointee will be expected to exercise leadership in teaching physiology and functional anatomy at the undergraduate and graduate level and to develop the research and investigative activities of the physiology area of the Department of Medical Technology.

Salaries: Senior Lecturer 1 $A22,289 to $A23,737; 11 $A20,365 to $A21,810. Appointment will be made in accordance with qualifications and experience.

Q.I.T. is a multi-disciplinary institution with an enrolment of approximately 7,000 students. Brisbane, the State capital of Queensland, has a population of around one million, and enjoys a climate similar to that of southern Spain.

Further information may be obtained from the Personnel Officer, Q.I.T., G.P.O. Bor 2434, Brisbane Q. 4001.

Applications quoting reference No. V.132/78, full details, telephone number together with a recent photograph should reach the Personnel Officer by December 16, 1978.

8448(A)

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

(University of Reading)

CHEMISTRY DEPARTMENT

Applications are invited for a scientist to participate in research into the functional properties of milk protein. The work will involve separations, identification and quantification of proteins, the enzymic and chemical modification of proteins and examination of their functional properties. Honours degree or equivalent in chemistry, biochemistry or food science is required. Some experience in protein chemistry would be advantageous.

Appointment will be as Scientific Officer (£2,839 to £4,415) with starting salary according to qualifications and experience. Non-contributory superannuation.

Application forms and further details are obtainable from the Secretary, N.I.R.D., Shinfield, Reading RG2 9AT. Quote reference 78/35. 8444(A)

The Division of Clinical Nutrition

THE HOSPITAL FOR SICK CHILDREN

Toronto, Ontario

is seeking applicants for, a position as a geographical full time staff member. The applicant should be a trained Paediatric Physician or Surgeon, with a demonstrated interest and competence in Nutrition Research. The position will entail, patient care, teaching and the conduct of original research, in the area of Human Nutrition. Applicants are requested to correspond with Dr P. B. Pencharz, Head, Division of Clinical Nutrition, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8. 8389(A)

UNIVERSITY OF EDINBURGH
RESEARCH TECHNICIAN

required to work in the
DEPARTMENT OF MOLECULAR BIOLOGY

on a project entitled 'Bacterial plasmids and conjugation' An Honours degree, or equivalent, in an appropriate subject is required. Salary on the scale £3,186 to £3,770 p.a. (under review). Appointment to take effect from January 1, 1979 for a period of five years. Ref. NO38.

Application forms for the above post can be obtained from the Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS. Telephone: 031-556 2930. 8434(A)

Food Chemist in Government Research (SSO/HSO/SO) £2839-£6898

Applications are invited from suitably qualified candidates for a pensionable post in the Agricultural and Food Chemistry Division of the Department of Agriculture situated at Newforge Lane, Belfast.

The duties will include research and advisory work in Food Chemistry snd the successful candidate may also be required to undertake lecturing duties in Food Chemistry and Allied subjects of the Honours course leading to the degree of BSc in Food Science at the Queen's University of Belfast.

Appointment may be at Senior Scientific Officer, Higher Scientific Officer, or Scientific Officer level.

SENIOR SCIENTIFIC OFFICER

Over 25 and under 32 years of age on December 31, 1978, and possess a 1st or 2nd class Honours Degree in Food Science, Chemistry, Biochemistry or Agricultural Chemistry and at least 4 years' appropriate post-graduate experience.

HIGHER SCIENTIFIC OFFICER

Under 30 years of age on December 31, 1978, and possess an Honours degree as above and at least 2 years' appropriate post-graduate experience.

SCIENTIFIC OFFICER

Under 27 years of age on December 31, 1978, with an Honours degree as above.

Preference may be given to candidates with appropriate research experience in Food Chemistry or experience in teaching Food Chemistry and the Chemistry and Physics of Food Processing.

Exceptionally applications may be considered from candidates over the age limits who have specialised experience.

The Commissioners may decide to interview only those applicants who appear from the information available (including level of academic attainments and experience) to be best qualified.

SALARY SCALES

Senior Scientific Officer ..	£5,154 to £6,898
Higher Scientific Officer ..	£4,101 to £5,448
Scientific Officer	£2,839 to £4,415

Grading and starting salary will be related to qualifications and experience.

Please write or telephone for an application form quoting job reference SB 406/78/N to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (telephone Dundonald 4585 ext 256). Completed forms must be returned to arrive not later than November 30, 1978.

8407(A)

NORTHERN IRELAND
CIVIL SERVICE

Colaiste na hollscoile Corcaigh

UNIVERSITY COLLEGE CORK

The Governing Body invites applications for the following office:

PROFESSORSHIP OF GEOLOGY

Salary range (from March 1, 1979) is: £9,775 to £11,305 p.a.

Application forms and further details may be obtained from the undersigned.

Latest date for receipt of applications is **Wednesday, December 6, 1978.** M. F. Kelleher, Secretary. 8462(A)

Laboratory Technicians

Hoechst UK Limited are looking for people to take up these challenging positions on their site at Walton, Milton Keynes.

There are currently two vacancies in the **Pharmaceutical Development Department.**

Analytical Section

The job consists of taking and analysing samples generated during the development of new pharmaceutical dosage forms. Raw materials, drug substances, pharmaceutical excipients, process investigation samples and finished products are tested using a variety of physical and chemical methods.

Formulation Section

The person appointed will assist in the development of pharmaceutical dose forms – tablets, capsules and liquid products – and prepare small scale batches of products for evaluation.

Candidates, male or female, for both these posts should have laboratory experience and be educated at least to 'A' level in Chemistry.

Hoechst UK is a part of one of Europe's leading companies and the salaries and conditions of employment are of the standard to be expected from such a company.

Please write or phone for an application form to:

The Personnel Department, Hoechst UK Limited, Walton Manor, Walton, Milton Keynes, Bucks. Tel: Pineham 5068.

8409(A)

Hoechst

Leeds

POLYTECHNIC

School of Health and Applied Sciences

LECTURER II IN NUTRITION AND DIETETICS

Applications are invited from State Registered Dietitians having clinical experience, to join a young and enthusiastic team concerned with the teaching of Nutrition and Dietetics to Degree and Diploma students in a well established school.

Salary Scale: £4,101 to £6,558.

Details from:
The Services Officer (ND30) Leeds Polytechnic,
Calverley Street, Leeds LS1 3HE. Tel: 0532 462923.

Informal enquiries may be made to Dr K. R. Fell, Head of School (Tel: 0532 463941).

Closing Date: December 1, 1978. Please enclose s.a.e.

8403(A)

POSTDOCTORAL POSITION

available in laser light scattering laboratory. Background in polymer solution chemistry or physical biochemistry required. Contact Professor F. E. Karasz, Department of Polymer Science and Engineering, or Professor N. C Ford, Department of Physics, University of Massachusetts, Amherst, 01003. An Equal Opportunity/Affirmative Action Employer 8466(A)

STRUCTURAL BIOLOGIST

The Department of Biological Sciences, Carnegie-Mellon University, invites applications from individuals whose research interests include the use of biochemical and biophysical techniques to study biological structures. The candidate should have post-doctoral experience. The position is at the assistant or associate professor level. Responsibilities include teaching at the undergraduate and graduate level, and carrying out an active research program The applicant should forward a curriculum vitae, three or more letters of reference, and a brief résumé of research plans to Search Committee (B), Department of Biological Sciences, Carnegie-Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213, U S A.

Carnegie-Mellon University is an Equal Opportunity/Affirmative Action Employer. Please respond by December 15, 1978. 8446(A)

UNIVERSITY OF SURRE

DEPARTMENT OF SOCIOLOGY

M.Sc. IN SOCIAL RESEARCH

Part-time

Applications are invited for t
1978–80 intake of the Diplom
M.Sc. course in Social Resear
which is held on FRIDAYS duri
term-time over two years. T
course, which most students atte
on a day-release basis, covers ma
aspects of research design and
cludes the analysis of bo
quantitative and qualitative
search material. It is intended f
those, from a wide variety of p
fessional fields, who need to und
take or evaluate social research.

All students take courses in d
analysis, research metho
organisational theory, and soc
policy, together with introduct
courses on the main concepts
sociology and social psycholo
In addition students may select c
of the following options:

Sociology of Education
Social Services and Social Work Research
Medical Sociology and Health Services Research
Urban Sociology and Community Research
Industrial Sociology

Entry is open to graduates a
others with professional traini
and relevant experience.

A leaflet describing the cou
and application forms are ava
able from the Registry, Univers
of Surrey, Guildford, Surr
GU2 5XH.

Closing date for applicatio
December 31, 1978.

8471(A)

ASSISTANT PROFESSO

OF BIOCHEMISTRY

Persons with an interest
developing a research program
some aspect of plant life releva
to crop productivity, a Ph.
degree, and demonstrated abili
to conduct biochemical
molecular biological research
invited to apply. Send curricul
vitae, statement of future resear
interest and names of thr
references by January 15, to. L
K. Miller, Department of Bacte
ology and Biochemistry, Univers
of Idaho, Moscow, ID 83843.
E O /A.A. Employer. 8405(A)

POPULATION BIOLOGI

The Department of Biology at
University of California at Rive
invites applications for a fac
position in population biology be
ning September 1, 1979. Candid
must show evidence of ability
initiate and sustain a productive, h
quality research program and
enthusiasm and skill in teachin
both undergraduate and grad
levels Teaching duties would inc
an introductory population bio
course and a graduate field cou
Appointment will be made at
Assistant Professor level.

Applicants should send curric
vitae, statement of teaching
research interests, reprints and le
from three referees.

The University of California i
equal opportunity and affirm
action employer. Applications
invited from all qualified individ
especially women and members
minority groups.

Send applications to:

Irwin W. Sherman, Chairma
Department of Biology
University of California.
Riverside, California 92521

8430(

UNIVERSITY OF EDINBURGH
DEPARTMENT OF GEOPHYSICS
POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the post of Postdoctoral Research Fellow to work on the analysis of regional gravity data in Britain. The project is collaborative with the Applied Geophysics Unit of the Institute of Geological Sciences and other University groups, and has the objective of preparing and interpreting a regional gravity map of Britain stripped of the contribution of upper crustal sources.

Applicants, who will need to be numerate with computing experience, should have a good background knowledge of British geology. Although based in Edinburgh, they should be prepared to spend part of their time working in collaborating institutions.

The appointment is for three years with a salary of £4,382 to £4,882 p.a. and immediate availability. Requests for further information and applications, including a full curriculum vitae and two referees, should be made to: Dr R. G. Hipkin, Department of Geophysics, J.C.M.B., Mayfield Road, Edinburgh EH9 3JZ. Please quote Reference 5064.

8424(A)

INSTITUTE OF TERRESTRIAL ECOLOGY
HIGHER SCIENTIFIC OFFICER

A Soil Scientist, geochemist or iemist, at Higher Scientific Officer :vel, is required at the Institute's esearch Station Bangor, Gwynedd, to udy the release and movement of fferent elements from minerals into id through soils to freshwater courses.

The research will form part of a ollaborative geochemical study with aff of the Institute of Geological :iences and the University College North Wales.

ualifications/Experience: Applicants iould have a good honours degree or uivalent in Soil Science or Geoiemistry and at least two years levant postgraduate experience in soil iemistry. An interest in experimental chniques and use of lysimeters would an advantage.

ilary, Conditions of Service: The ppointment will be to the Higher :ientific Officer grade. Starting salary cording to age, qualifications and perience on the following scale—,101 to £5,448 per annum, 22 days id annual leave plus 10½ days public id privilege holidays. There is a nonntributory superannuation scheme. aff of the NERC are not Civil rvants but their pay and conditions service are similar to those of the vil Service.

Application forms and further tails are available from:

Establishments Section
Institute of Terrestrial Ecology
68 Hills Road,
Cambridge CB2 1LA
Tel: (0223 69745) quoting ref. 357

The closing date for completed plications is December 7, 1978.

'HE NATURAL ENVIRONMENT RESEARCH COUNCIL

8494(A)

STITUTE OF Cancer Research—boratories at Sutton, Surrey. Rearch Biologist required to join oratory group investigating biology human leukaemia; laboratory exrience in tissue culture essential. ie appointment is for a three year riod initially. Salary according to perience in range £3,261 to £4,680 a. Applications in duplicate with the mes of two referees to the Secretary, stitute of Cancer Research, 34 mner Place, London SW7 3NU, oting ref. 301/B/54. 8452(A)

THAMES POLYTECHNIC
SCHOOL OF MATERIALS SCIENCE AND PHYSICS
X-RAY and NUCLEAR LABORATORY TECHNICIAN GRADE 5

Applications are invited for a Technician for the X-ray and nuclear laboratories who will be required to operate X-ray equipment, handle radioactive sources and be responsible for keeping exposure records. Candidates should have a recognised apprenticeship or HND/HNC qualification and at least eight years' relevant experience.

Salary scale: £3,474 to £4,011 inclusive.

Further particulars and form of application may be obtained from the Staffing Officer, Thames Polytechnic, Wellington Street, London SE18 6PF, to whom completed applications should be returned by November 28, 1978. 8496(A)

CENTRAL PUBLIC HEALTH LABORATORY
National Collection of Type Cultures
SYSTEMS DESIGNER/ PROGRAMMER

A science graduate is required for the computer operation of a bacterial identification service. Bacteria from clinical sources are identified by probabilistic analysis of test results by reference to computer-stored data bases. Systems use Optical Mark Reader forms, Data Recognition (Dataterm 3) Machine, Extel punch tape terminal connected to the LUCS CDS 6500 time sharing system; current software in FORTRAN IV. There is considerable scope for improving the responsiveness of the system and the efficient storage and retrieval of data, and to revise the methodology or explore alternative methods.

Candidates with five years postgraduate experience may be appointed to the Senior Scientist Grade.

Salary on probationary scale £2,991 to £4,275 plus London Weighting, Post Probationary scale £3,798 to £4,899 plus London Weighting or Senior Scientific scale, if applicable, £5,451 to £6,837 plus London Weighting.

Ring 01-205 7041 for a more detailed job description (Dr L. R. Hill, on extn. 47) and for conditions of employment and application form (Personnel Officer, on extn. 46). 8470(A)

ROTHAMSTED EXPERIMENTAL STATION
Harpenden, Herts. AL5 2JQ
SCIENTIFIC OFFICER

to work in the Entomology Department insect survey. The work will consist of sorting and identifying aphids, curating and compiling records, with opportunity for ecological studies. Some knowledge and experience of aphid taxonomy is desirable.

Qualifications: Honours Degree or equivalent in Zoology, Agricultural Entomology or Applied Entomology, or Pass Degree, H.N.C., or equivalent with relevant post qualifying experience.

Appointment in grade of Scientific Officer (£2,839 to £4,415 per annum). Non-contributory superannuation.

Apply in writing to the Secretary, giving names and addresses of two referees, and quoting Ref. 370 by December 8, 1978. Further details on request. 8428(A)

ICI

Research Biochemist

ICI's Corporate Laboratory has a vacancy for a research biochemist, with experience of preparative and analytical enzymology, and some knowledge of oxidation and reduction in biological systems and lipid biochemistry.

The successful applicant will become a member of an interdisciplinary team studying the industrial applications of enzymes and membranes and will need to be able to interact effectively with people of all levels, both within and outside ICI. It is hoped that he she will develop his/her own line of research within the general remit of the team.

A good honours degree or equivalent in biochemistry, coupled with relevant postgraduate experience are desirable qualifications for the post.

Please write, enclosing full curriculum vitae to:

Mrs C. Cleave
Personnel Assistant
ICI Corporate Laboratory
PO Box No 11
The Heath
RUNCORN Cheshire WA7 4QE

8426(A)

Culham
Laboratory

require a

PHYSICS GRADUATE

to work in the general field of particle throughput and radiation energy loss in magnetically confined plasma devices.

Current work involves critical assessments of the needs for atomic data coupled with measurements of cross-sections using colliding beam techniques.

Post graduate experience preferably in a related area of experimental physics is required.

Salary: £4,388-£7,380 (according to age, qualifications and experience).

For an application form write to (or telephone):

Mrs Diana Townsend
Personnel Department
UKAEA
Culham Laboratory
Abingdon
Oxon OX14 3DB
Telephone No.: 0235 21840 ext. 250

8421(A)

Liquid Chromatography . . .
. . . science with a sales bias

Du Pont (U.K.) Ltd., one of the world's largest chemical companies, are expanding their share of the liquid chromatography market.

This new appointment, in their Instrument Product Division at Hitchin, is the link beween Du Pont's introduction of new instrumentation and columns and the continuously expanding market for this sophisticated technology.

You will arrange and develop customer demonstrations, lectures and seminars, in the UK and occasionally abroad, in support of your own sales programme for new and existing customers and ensure effective after-sales service through field engineers.

Ideally, you will be around 30 with graduate or postgraduate qualifications in chemistry, or related fields, and some experience in the selling and marketing of HPLC would be an advantage.

However, we would also invite applications from recent graduates or postgraduates in the above fields who in their first jobs have demonstrated the personality, determination and drive to develop into a successful sales role.

This represents a real opportunity to develop in both professional and personal terms within an acknowledged market leader. The rewards are an above-average salary, regularly reviewed, prestige company car, pension plan and relocation package.

Please telephone (01-629 1844 at any time) or write for information and an application form, quoting ref. V.382. **ASL Recruitment Advertising, 17 Stratton Street, London W1X 6DB.**

DU PONT **Instrument Products**

8475(A)

Experimental Officer – Microbiologist

There is a vacancy for a microbiologist in a small team involved in the discovery of new fungicides and bacteria for agriculture. The job-holder will provide cultures of plant pathogens for use in glasshouse and field experiments, and will also conduct mode-of-action studies on fungicides at the morphological/physiological level. This job will suit a person with academic training to first degree or HNC level. Research experience in some aspect of microbiology is essential.

Jealott's Hill Research Station is situated on a pleasant farm estate between Bracknell and Maidenhead.

We offer good salaries and fringe benefits include membership of the ICI Profit Sharing Scheme and Pension Fund. A staff restaurant and recreation club are on site. Subsidised coaches run to and from Jealott's Hill, Bracknell and Maidenhead.

If you are interested in this vacancy, please write for an application form to:

Mr S R Stephenson
Personnel Officer
ICI Plant Protection Division
Jealott's Hill Research Station
Bracknell
Berkshire

Please quite reference number 78/EO Micro Bio.

ICI Plant Protection Division

8438(A)

WOLFSON UNIT OF
Plant Cell Biotechnology
University of Sheffield

The Wolfson Foundation has provided funds to establish a Unit of Plant Cell Biotechnology, based on the Departments of Biochemistry (Dr. M. W. Fowler) and Chemical Engineering (Professor J. A. Barnard), to develop the large-scale culture of plant cells as sources of pharmaceuticals and related chemicals. In addition to its own experimental programme, the unit will maintain close and continuing links with industry.

Vacancies exist for **TWO RESEARCH ASSISTANTS,** men or women, preferably at the post-doctoral level.

One will be concerned with the growth of plant cells in large-scale liquid culture. A knowledge of secondary product metabolism and experience of sterile techniques would be an advantage.

The other will be concerned with biochemical reactor design and scale-up to pilot plant and eventually industrial scale production. Experience of chemical engineering in the pharmaceuticals or related industries would be valuable.

In both cases, staff will be expected to collaborate closely with industry and to foster co-operation between the Unit and outside research organisations.

Salaries on the Research Assistant 1A scales £3,883 — 6,555, according to qualifications and experience. Appointments would be initially for one year with possible renewal.

Those interested should write in the first instance to:

Professor J. A. Barnard,
Department of Chemical Engineering
and Fuel Technology, University of Sheffield,
Mappin Street, Sheffield, S1 3JD
Quoting ref: R212/G 8401(A)

pcb

JUNIOR FACULTY POSITION
DEPARTMENT OF ASTRONOMY AND STEWARD OBSERVATORY, UNIVERSITY OF ARIZONA

The Department of Astronomy and Steward Observatory expects to make an appointment at the Assistant Professor tenure track level beginning the fall semester of 1979. Applicants in any area of astronomy and and astrophysics will be considered; some preference will however be given to applicants with interests in theoretical work or in infrared and optical observations. Facilities available include the 4.5m Multi-Mirror Telescope, 2.3 and 1.5 meter telescopes, and the University's CDC Cyber 175 and Dec 10 computers. Interested applicants are requested to send a vitae and biblography by February 15, 1979 to Dr Rodger I. Thompson, Chairman: Search Committee, Steward Observatory, University of Arizona, Tucson, Arizona 85721. The University of Arizona is an Equal Opportunity/Affirmative Action Title IX/Section 504 Employer.

8419(A)

Fermentation Process Development

Paris

Roussel Uclaf, the major international Group in the chemical and pharmaceutical industries, is seeking a Biochemical Engineer for its Research Centre located near Paris where the bulk of the Group's research work is carried out

As the Section Head, Fermentation Process Development, reporting to the Biochemical Development Manager, you will be responsible for

- new product process development, particularly of new techniques for fermentation processes and product recovery,
- process maintenance and improvements related to the production of existing fermentation products

Ideally, you should have a PhD in a relevant discipline and experience in those aspects of microbiology, biochemistry, chemistry and biochemical engineering necessary for the effective management of a team operating in this field.

Excellent conditions of service include a very competitive salary, contributory pension and free life insurance. Assistance with relocation will be given

Please send full career details to M R de Vitry, Direction du Developpment et de la Gestion des Cadres, Roussel Uclaf, 35 Boulevard des Invalides, Paris 7e, France

ROUSSEL

8481(A)

JOHN INNES INSTITUTE

DEPARTMENT OF GENETICS

Postdoctoral Fellow required to work on the molecular biology of crown-gall disease induced in plants by *Agrobacterium tumefaciens* Applicants should have, or expect shortly to obtain, a Ph D. in biochemistry, molecular biology or a related field. This post, funded by a grant from the Cancer Research Campaign, is for three years.

Salary will be at an appropriate point on the Higher Scientific Officer scale (£4,101 to £5,448) Non-contributory superannuation

Further particulars may be obtained from the head of the Genetics Department, Professor D. A. Hopwood Applications quoting ref. G/28 with full details of qualifications and experience and the names of two referees, should be sent to the Secretary, John Innes Institute, Colney Lane, Norwich, NR4 7UH

8485(A)

UNIVERSITY OF GLASGOW

LECTURESHIP IN BOTANY

Applications are invited from experimental biologists for a Lectureship in Botany. No field of research interest is excluded but the work of applicants trained in fields other than Botany (e.g. Virology, Molecular Genetics, Environmental Physics, etc.) must be associated with plant tissues after appointment The appointment will begin as soon as possible and no later than October 1, 1979. The initial salary will be £3,883 to £5,604 (interim) on the Lecturers' scale

Further information about the post and department may be obtained from Professor M B Wilkins.

Applications (8 copies), giving the names and addresses of three referees, should be lodged with the Secretary of the University Court, (Room 18), The University of Glasgow G12 8QQ, not later than December 31, 1978

In reply please quote Ref. No. 4111/1M. 8302(A)

FELLOWSHIPS

AUSTRALIAN NATIONAL UNIVERSITY

RESEARCH SCHOOL OF EARTH SCIENCES

RESEARCH FELLOWSHIPS (3 Posts)

Applications are invited for appointment to the following :

RESEARCH FELLOW IN GEOPHYSICAL FLUID DYNAMICS

Since the appointment of Professor J. S. Turner in 1975, the Geophysical Fluid Dynamics Group has worked in two main research areas: experimental and theoretical studies of mixing and convection processes in the ocean, and theoretical work on the interior of the earth and other planets. Inter-disciplinary research with other groups has also been initiated, in particular a study of the fluid phenomena associated with the formation of ore bodies. The group has a well-equipped laboratory, and has just acquired a DEC Computer for data logging and analysis.

The successful applicant will have considerable freedom to pursue his/her own research within the broad field of fluid geophysics Preference will be given to candidates who have some experience in both theoretical and laboratory modelling, and have demonstrated the ability to relate their results to observational data.

RESEARCH FELLOW IN ISOTOPE GEOLOGY

Candidates for this post should be geologists or geochemists who are trained in the use of isotopes in earth sciences. The successful applicant will join the Isotope Group within the R.S E.S. and conduct research using terrestrial or meteoritic materials in one or more fields of current interest. Present facilities include K-Ar, Rb-Sr and U-Pb age determinations and carbonate C, O isotopic analyses; the use of other isotopic methods is under consideration

RESEARCH FELLOW IN SEISMOLOGY

The School has an active ongoing research program in determining the seismic structure of the mantle under the Australian continent using fixed and mobile arrays. Applicants should be seismologists interested in such studies or in related fields. This would include theoretical seismologists interested in applying synthetic seismogram theory to mantle models or interested in source mechanisms. Applications from geophysicists with broader interests in the structure of the interior of the Earth and terrestrial planets, in investigations of upper mantle lateral heterogeneity, or in tectonic problems in the Australasian region will also be considered for this position. Exceptionally well qualified candidates may be considered for appointment at Senior Research Fellow level.

CLOSING DATE: **JANUARY 31, 1979.**

SALARIES. Salary on appointment will be in accordance with qualifications and experience within the ranges: Senior Research Fellow $A21,201 to $25,289 per annum, Research Fellow $A15,179 to $19,813 per annum Current exchange rates $A=$US1.17:58p.

Appointment as Senior Research Fellow or Research Fellow will be for three years in the first instance with the possibility of extension to five years.

OTHER CONDITIONS: Reasonable appointment expenses are paid Superannuation benefits are available for applicants who are eligible to contribute Assistance with housing is provided for an appointee from outside Canberra.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should obtain further particulars from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF 8455(E)

POSTDOCTORAL FELLOWSHIP

(One)

relating to Huntingdon's Chorea in either preclinical sciences, including biochemistry, physiology, pathology or pharmacology, or from such clinical specialities as neurology, psychiatry or human genetics. M D. or Ph.D. required. Initial stipend $13,000 and upward, dependent on degree and previous postdoctoral experience. Deadline is February 1, 1979. To be announced by April 1, 1979. The Fellowship will begin July 1, 1979 and is to be held for one year with renewal for a second year, dependent on progress. Write Huntington Society of Canada, Box 333, Cambridge, Ontario, Canada NIA 5T8 for application and instructions Preference will be given to Canadians, or landed immigrants to Canada 8483(E)

Postdoctoral Fellowship in ENZYMOLOGY

TRENT UNIVERSITY CANADA

Postdoctoral position available early in 1979 for enzymologist to investigate biosynthesis of coumarins. Experience with plant systems desirable but not essential Send curriculum vitae and names of two referees to Dr S. A Brown, Department of Chemistry, Trent University, Peterborough, Ont., Canada K9J 7B8. 8387(E)

POSTDOCTORAL FELLOWSHIP

Two three-year postdoctoral fellowships are available at the Friedrich Miescher-Institut, Basel, in a group working on several aspects of human interferon.

BIOCHEMIST

Preferably experienced in protein purification and/or protein chemistry. The research will involve production and purification of human lymphoblastoid interferon, its characterisation and mode of action. Experience in mammalian tissue culture methods would be of advantage but is not essential.

MOLECULAR BIOLOGIST

Experience in RNA and DNA metabolism is essential. The project will include mRNA isolation, *in vitro*—translation and the cloning of DNA sequences in bacterial plasmids

Reply to Dr H. Weideli, The Friedrich Miescher-Institut, P O Box 273, CH-4002 Basel, Switzerland, sending a brief curriculum vitae and names of three referees. 8487(E)

NEW HALL, CAMBRIDGE

New Hall invites applications for a College Lectureship (with Fellowship) in Physiology from October 1, 1979 Further particulars may be obtained from the President, New Hall, Cambridge CB3 0DF. The closing date for applications is December 16, 1978.

8465(E)

FELLOWSHIPS

UNIVERSITY OF EDINBURGH
Postdoctoral Fellowship on the Molecular Biology of Papilloma Viruses

Applications are invited for a Fellowship for work on a joint project in Edinburgh and Glasgow Universities The project is to investigate the state of bovine papilloma viral DNA in cattle warts and tumours The physiological aspects of the project—including growth of virus and tumours, and transformation of cells—will be conducted at the Veterinary Pathology Department at Glasgow University, directed by Professor W F H Jarrett. The molecular aspects, with which the Fellow will be mainly involved, will be carried out in the Zoology Department at Edinburgh University directed by Drs M. S Campo and U. E Loening The Fellow should be skilled in the current techniques of molecular biology such as hybridisation and restriction enzyme analysis of nucleic acids.

The post is financed by an Agricultural Research Council grant to Professor Jarrett, and the salary is on the Research Range 1A scale The Fellowship can start immediately.

Applications should be sent to Drs M S Campo and U E Loening, Department of Zoology, West Mains Road, Edinburgh 3JT by **January 10, 1979.** Please quote Reference 5068 8458(E)

UNIVERSITY OF EDINBURGH
DEPARTMENT OF BIOCHEMISTRY
POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for the above post supported by the Medical Research Council and tenable for 2 years from April 1, 1979 The project will investigate the biological significance of cytochrome c methylation and the use of methylation as a means of studying protein . protein interactions Some experience in protein chemistry or enzyme purification is desirable Starting salary in the range £3,883 to £4,631 p a

Further information may be obtained from Dr G W Pettigrew, Veterinary Unit, Department of Biochemistry, Royal (Dick) School of Veterinary studies, Summerhall, Edinburgh EH9 1QH, to whom applications including the names of three referees should be sent before December 31, 1978

In reply please quote Reference 5067 8449(E)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF MICROBIOLOGY
RESEARCH FELLOWSHIP

A postdoctoral research fellow required for studies on the pathogenicity of influenza virus supported by the Medical Research Council Virological training preferred but not essential The post is for a Research Fellow 1A in the range £3,883 to £6,555 (plus superannuation), beginning January 1, 1979 for two years and nine months Maximum starting salary will be £4,882

Applications should be sent to the Assistant Registrar, Science and Engineering, University of Birmingham, P O Box 363, Birmingham B15 2TT by Friday December 8, 1978, and further details can be obtained from Professor H. Smith

Please quote ref. NM4. 8439(E)

POSTDOCTORAL FELLOWSHIPS
DEPARTMENT OF PHYSICS
UNIVERSITY OF WATERLOO
WATERLOO, ONTARIO, CANADA

Applications are invited for postdoctoral research fellowships in physics.

Fellowship stipends range between $11,100 and $12,900 and will depend on the teaching duties. The awards are tenable for the period of one year with a possible renewal for a second year

Research areas include:

Atomic and Molecular Physics, Biophysics, Brillouin Spectroscopy, Crystallography, Electron Microscopy, Electron tunneling, Ellipsometry, Experimental superconductivity, Diffraction Physics, Far-infrared spectroscopy, Infrared and Raman Spectroscopy, De Haas-van Alphen effect, Laser Physics, Lattice dynamics, Magnetoconductivity of metals, Microwave Collision-Induced Absorption and Interferometry, Nuclear magnetic resonance, Semiconductors and amorphous semiconductors, Transport properties of gases and intermolecular potentials, Theoretical studies; magnetic impurities in superconductors, relativistic effects in metals, electronic structure in metals, nonlinear behaviour of elastic dielectrics, lattice dynamics of ionic crystals

The applicants should submit their resumes and the names of three referees to:

Dr C C Lim
Department of Physics
University of Waterloo
Waterloo, Ontario, Canada
N2L 3G1 8391(E)

CALIFORNIA INSTITUTE OF TECHNOLOGY
RESEARCH FELLOWSHIP IN RADIO ASTRONOMY

Caltech's Owens Valley Radio Observatory invites applications for a one-year Research Fellowship in Radio Astronomy This position is associated with the development of a millimeter-wave interferometer, and applicants should have experience in relevant areas such as millimeter-wave spectroscopy, interferometry or instrument design and development and be prepared to devote at least half-time to this work There will also be opportunities for independent research in galactic or extragalactic radio astronomy and for cooperation with other astronomical groups in Pasadena One 10m telescope for millimeter-wave astronomy is now in operation, additional telescopes and low-noise receivers are under construction The appointment is renewable for one or two additional years

Prospective research fellows must complete their Ph D before beginning this appointment Application forms may be obtained from, and should be returned by February 1, 1979, to Professor Alan T Moffet, Director, O V R O Caltech 102-24, Pasadena, CA 91125, U S A

Caltech is an equal opportunity affirmative action employer

8418(E)

POSTDOCTORAL FELLOWSHIP

Available from January 1, 1979 for a Ph D graduate in biochemistry, virology or related area interested in studying the structure and replication of mammalian picornaviruses Applicants must have received the Ph D degree no earlier than April 1, 1978 Salary is $11,600 for the first year

Send curriculum vitae and references to Dr D G Scraba, Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7 Canada 8388(E)

THE QUEEN'S UNIVERSITY OF BELFAST
RESEARCH FELLOWSHIP
DEPARTMENT OF MICROBIOLOGY AND IMMUNOBIOLOGY

This post involves work on macromolecular aspects of neurological disease Preference will be given to candidates with experience in virology, immunology, particularly in immuno-assay methods and/or nucleic acid hybridisation techniques The Fellowship is tenable for two to three years at a commencing salary of £4,134 per annum with F S S U or U S S

Application forms may be obtained from the Personnel Officer, The Queen's University of Belfast BT7 1NN, Northern Ireland Closing date December 15, 1978 8459(E)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND
GEOPHYSICS AND PLANETARY PHYSICS

Applications are invited for a POSTDOCTORAL FELLOWSHIP or RESEARCH FELLOWSHIP in small, friendly research group with wide interests in theoretical global geodynamics and planetary physics geophysical fluid dynamics, planetary magnetism, rotation, deformation Appointment will be for one year in the first instance, renewable for a second year Send curriculum vitae and names of three referees to Professor M G Rochester, Physics Department, Memorial University of Newfoundland, St John's, Newfoundland, Canada A1B 3X7. 8400(E)

POSTDOCTORAL FELLOW

Laboratory with active program in the study of hormone receptor/antibody interactions has an opening for an individual with experience in hormone receptor separation and purification Although funds for the position at the M R C Canada Postdoctora Fellowship rate of $13,000 are available, the successful candidate would be encouraged and assisted in applications for his/her own Fellowship support Applications should include curriculum vitae and previous research experience The applicant should request three referees to send letters of support to: Dr N R Farid, Faculty of Medicine, Health Sciences Centre, 300 Prince Phillip Drive, St John's, Newfoundland A1B 3V6 8467(E)

UNIVERSITY OF DUNDEE
DEPARTMENT OF MATHEMATICS
RESEARCH FELLOWSHIP

Applications are invited for a Research Fellowship to undertake theoretical investigations in acoustics and electromagnetism Applicants should have appropriate qualifications in Mathematics, Physics or Engineering but need not have worked in the field of acoustics or electromagnetism previously

The starting salary is £3,895 per annum (under review) with U S S. or continuation or F.S S U The appointment will be available from an early date to be agreed for a period of one year in the first instance, with a possibility of subsequent renewal

Applications should be sent by **December 7, 1978** to The Secretary, The University, Dundee DD1 4HN from whom further particulars and further information can be obtained Please quote Reference EST/55/78J. 8416(E)

STUDENTSHIPS

THE ROYAL SOCIETY
John Murray Travelling Studentship in Oceanography and Limnology 1979

Applications are invited for the above Studentship, open to British graduates under the age of 35, for the encouragement of travel and work in oceanography or limnology This award, which may be held for periods from three months to one year, will in 1978 be up to £1,500, adjusted according to qualifications and place and duration of research Applications are especially invited from workers in oceanography and limnology, as well as in zoology, botany, geology, mathematics 8406(F)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
DEPARTMENT OF MEDICAL PHYSICS
RESEARCH STUDENTSHIP

Applications are invited for a Research Studentship (£1,990 p a plus approved fees), tenable for 3 years for experimental studies in ultrasonic diagnostic techniques. The student will be expected to register for a higher degree Applicants should preferably have some experience in computer programming

Applications, with curriculum vitae and the names of two referees, to Professor H A B Simons, Department of Medical Physics, Royal Free Hospital, Pond Street, London NW3 2QG. 8385(F)

MICROBIOLOGY graduate required for a Ph D. Studentship in connection with a research project on bacteriological aspects of acne. Apply in writing, with full curriculum vitae and the names of two referees, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref M C.6 8422(F)

ASSISTANTSHIPS

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY
DEPARTMENT OF GEOLOGY
POSTDOCTORAL RESEARCH ASSISTANT
DEEP GEOLOGY PROJECT

Applications are invited for two postdoctoral research assistanships to be taken up as soon as possible. These posts form part of a three year study of the deep geology of Southern England in collaboration with the Institute of Geological Sciences Applicants should be stratigraphers with a broad knowledge of geology and geophysics and an ability to work with experts in other fields A good knowledge of French is an advantage Salary within range £4,359 to £5,579 including London Allowance and USS

Further particulars can be obtained from Dr P Wallace, Department of Geology, Imperial College, London SW7 2BP, to whom applications including curriculum vitae and names of two referees should be sent Closing date December 8, 1978 8491(F)

GRANTS

THE ROYAL SOCIETY
Browne Research Fund
Maurice Hill Research Fund
Marshall and Orr Bequest
GRANTS 1979

The Council of the Royal Society invites applications from qualified research workers for personal grants

(a) from the Browne Research Fund, for research in marine biology at a marine biological laboratory or elsewhere. Preference will be given to those who propose to work on purely scientific problems rather than to those whose work would be primarily directed towards an economic end,

(b) from the Maurice Hill Research Fund (established in memory of the late Dr M. N. Hill, F.R.S., for the encouragement of research in physical oceanography and marine geophysics) for research at sea and/or in the laboratory in physical and chemical oceanography, marine geophysics and geology.

(c) from the Marshall and Orr Bequest for the support of marine biological research

Grants may be used for periods up o one year, and are made on the nderstanding that university or other osts held by applicants will meanwhile be kept open for them ecipients are permitted to use grants hile receiving salaries from regular ppointments.

In 1979 the total amounts available o be shared among all successful pplicants will be about £4,250 from he Browne Research Fund, £8,400 rom the Maurice Hill Research Fund nd £7,820 from the Marshall and Orr equest; individual grants will not rdinarily be more than £750.

Applications should be made on orms to be obtained from the xecutive Secretary, The Royal ociety, 6 Carlton House Terrace, ondon SW1Y 5AG, and returned by ecember 15, 1978. 8398(E)

COURSES/CONFERENCES LECTURES/SYMPOSIA

European
Molecular Biology
EMBO COURSE
ION-SENSITIVE INTRACELLULAR MICROELECTRODES
Bristol, England
June 25—July 8, 1979

The course is for 12 active esearchers who wish to learn how to se ion-sensitive intracellular microelectrodes. Participants will be taught ow to make, calibrate and do experiments with K⁺, Cl⁻, Na⁺ and pH⁺-ensitive microelectrodes. Most of their me will be spent in the laboratory, lthough there will be some lectures uring the course and a two-day ound-table discussion at the end. uring the round-table, a number of ading workers in the field will escribe their methods and recent sults Instructors will include Roger homas, Dave Ellis, Richard Vaughan-ones, Jochen Deitmer, Bill Moody nd Andrew Sharp.

Applicants should have some experience of electrophysiological techniques, nd have a convincing reason for aking the course. Applications should nclude a brief curriculum vitae ason for taking the course and a tter of recommendation.

Acceptance for course will be otified to applicants early in March articipants will have to pay their wn accommodation and subsistence penses (estimated at about £100), eir travel expenses and a Course egistration Fee of £30. One or two ants are available for applicants who an pay only their travel expenses ccommodation will be arranged by e course organiser.

Applications to Dr R. C. Thomas. epartment of Physiology, University Bristol, Bristol BS8 1TD, England, ust be received by February 19, 79. 8480(D)

COURSES/CONFERENCES/LECTURES/SYMPOSIA

FEBS ADVANCED COURSES

No. 58, "Biomolecular Electron Microscopy"
Ulm, F.R.G.,
29 March–7 April 1979

The Course, organised by A. K. Kleinschmidt, is intended to offer information on selected methods of biomolecular structure studies, and laboratory instruction in electron-microscopic methods of macromolecular analysis, to 40 qualified applicants Specialised fields, e.g electron microscopy of nucleic acids, membrane structure, nucleoprotein particles, immunocytochemistry, lipoproteins and proteoglycans, and autoradiographic procedures and image analysis methods will be included to participants' choice in laboratory practical sessions. Course fee, including accommodation and food: approx DM400.—Applications (by January 1, 1979) and info: Dr A K Kleinschmidt, University of Ulm, Department of Microbiology, Postfach 4066, 7900 Ulm, F.R G.

No. 60. "Gene Structure and Formation of RNA of Viruses and Animal Cells"
Israel, 15–21 May 1979

A workshop organised by Y. Aloni and co-sponsored by E.M.B O. Fee: $280.—Info· Mr Y. Berman, Congress Section, Weizmann Institute of Science, Rehovot, Israel

No. 62. "Genome Organization and Expression in Higher Plants"
Edinburgh, Scotland,
11–21 July 1979

A course for up to 125 participants Accommodation including full board, £120 approx Info. Dr C. Leaver, Dept. of Botany, The King's Buildings, Mayfield Road, Edinburgh EH9 3JH, Scotland

No. 56. "Membrane Proteins"
Berne, Switzerland,
10–22 September 1979

The course, co-sponsored by the Swiss Committee for Molecular Biology and organised by P. Zahler and A. Azzi, will consist of 1 week each of lectures and experimental work, and discussions The course will concentrate on specific problems of membrane proteins (solubilization, fractionation, isolation, characterisation, reconstitution). Places will be restricted to 80–100 for the lectures and to 40 advanced participants for the experimental part Fees (including registration, accommodation and food): Sw Fr 700 —for the full course or Sw Fr 340 —for the lecture course only. Applications should be addressed before April 1, 1979 to: Professor P. Zahler, Institut fur Biochemie, Universitat Bern, Freiestrasse 3, CH-3012 Bern, Switzerland.

No. 57. "Genetic Engineering"
Zagreb, Yugoslavia,
8–24 October 1979

Info: Dr V Šlamić, Institut "Ruder Boskovic", Post. pret. 1016, 41001 Zagreb, Yugoslavia. 8464(D)

FIFTH INTERNATIONAL SYMPOSIUM ON GLYCOCONJUGATES

A small number of grants are available to aid younger scientists resident in the U.K. to attend the above meeting to be held in Kiel, G.F.R., from September 2-8, 1979. For full information about the meeting and enquiries concerning the travel grants please write in the first instance, to either Dr R C. Hughes, National Institute for Medical Research, Mill Hill, London NW7 1AA or Dr R D. Marshall, Department of Chemical Pathology, St Mary's Hospital Medical School, London W2 1PG. Applicants should indicate briefly (100 words) their research interests. 8468(M)

INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE FRANCAISE
EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

MOLECULAR AND CELLULAR ASPECTS OF OBESITY

AUGUST 27-29, 1979
NICE, FRANCE

The workshop is intended to bring together scientists actively working on different aspects of this topic. The number of participants is limited to about 60.

Sessions

1—Differentiation in culture of mammalian cells into adipocytes

2—Control of fatty acid synthesis and esterification in hepatocyte and adipocyte

3—Control of lipolytic activities in adipocyte.

Speakers (preliminary list)

G. AILHAUD (Nice)
A. W ALBERTS (Rahway)
P. BELFRAGE (Lund)
R. M. BELL (Durham)
P. BJORNTORP (Goteborg)
D. N. BRINDLEY (Nottingham)
R. M. DENTON (Bristol)
H. GREEN (Cambridge—USA)
D. LANE (Baltimore)
T OLIVECRONA (Umea)
D. A K. RONCARI (Toronto)
O. ROSEN (New York)
T. RUSSELL (Miami)
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Communication and Poster Sessions

An invitation to submit communications or posters is extended to all prospective participants.

Applications

Applications may support their applications by including one or more of the following: a list of recent publications, an abstract for a communication or for a poster, a brief summary of the applicant's interests in this area. Applications should be sent before April 15, 1979 to:

Dr G. AILHAUD
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Applicants will be notified of acceptance by May 31, 1979.

Registration

There will be no registration fee. The organization will cover accommodations and full board Sessions, meals and lodging will be at College International de Sophia-Antipolis (Nice). 8484(K)

IMPERIAL CANCER RESEARCH FUND DNA TUMOR VIRUS MEETING 1979
CHURCHILL COLLEGE CAMBRIDGE, ENGLAND
July 16-20, 1979

The Meeting will be on the molecular biology of small DNA tumour viruses, particularly polyoma, SV40 and adenoviruses and is open to anyone interested in the subject. The scientific programme will include oral presentations and posters.

The cost for registration, accommodation, food and the abstract book will be approximately £100. Travel money can only be provided in a few, very exceptional circumstances.

Anyone interested in attending the meeting should apply immediately for further details to 'The ICRF DNA Tumour Virus Meeting Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, England.

8360(C)

A NATO SCHOOL
on
TOPICS IN THEORETICAL ORGANIC CHEMISTRY

will be held in Gargnano (Garda Lake), Italy on June 18-30, 1979

Lectures will be delivered by.

A. Bernardi (Italy);
E. Clementi (Italy);
I. G. Csizmadia (Canada);
N. Epiotis (U.S.A.);
P. von R Schleyer (Germany)

Intending participants should contact.
Prof. A. Mangini, NATO School,
Istituto Chimica Organica,
Università,
Viale Risorgimento 4,
40136 Bologna, Italy 8488(C)

COURSES AND CONFERENCES
Continued on next page

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A
MEETING
ON
RECOMBINANT DNA

Organised by the Committee on Genetic Experimentation (COGENE) of the International Council of Scientific Unions and the Royal Society of London and will be held at Wye College, Kent, from 1-4 April 1979. The purpose of the meeting is to review the current status of recombinant DNA research and technology and to discuss matters of concern such as support for the research, its regulation through guidelines and legislation, and the eventual practical application of the technology.

Representatives of national recombinant DNA committees and other involved national and international organisations are being invited but a small number of places are being reserved for general applicants.

The registration fee for the Conference is US $80 or £40. The charge for accommodation and full board is US $120 or £60.

Those wishing to participate should write for a registration form and further details to The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, England.

8446(C)

NATO INTERNATIONAL SUMMER SCHOOL
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SEPTEMBER 2-14, 1979—PORTUGAL

Lectures and discussions **in English** will cover the following topics:

(1) Evaluation of experimental systems to transfer macromolecules into animal and plant cells.

(2) Genome organisation: (i) Expression of cloned DNAs injected into oocytes (ii) Genetic engineering in plant cells.

(3) Fidelity of transcription and translation: (I) Injection of DNAs, RNAs and tRNAs into oocytes and somatic cells

(4) Cell transformation and tumorigenicity: (i) SV40 model; (ii) In vitro traits that correlate with transformation.

(5) Transfer of nuclei and chromosomes into whole cells and cell hybridisation: (i) Mapping of eukaryotic genes; (ii) Control of cell differentiation.

(6) Targeting of drugs and macromolecules with liposomes

The lecturers will include: M. L. Birnstiel (Switzerland), M R. Capecchi (USA), J. E. Celis (Denmark), T. Ege (Sweden), G. Gregoriadis (England), A Grässmann (West Germany), G. Marbaix (Belgium), A. Loyter (Israel), D. Papahadjopoulos (USA), M Rechsteiner (USA), F. H Ruddle (USA), J. Schell (Belgium, J V. Small (Austria), D W. Stacey (USA), K. Willecke (West Germany), J. Zeuthen (Denmark).

The summer school will be suitable for predoctoral students and for postdoctoral workers in related fields.

Applications should be sent to Dr J E. Celis (Deparment for Biostructural Chemistry, Intitute of Chemistry, University of Aarhus, Langelandsgade 140, 8000 Aarhus C, DENMARK) no later than March 1, 1979 They should contain a curriculum vitae, including the applicant's scientific background and interests, and one letter of recommendation. There is no special application form.

The applicants will be informed before April 15, 1979, whether they have been accepted. An accommodation fee of US$125 will then be required. Participants will have to cover their own travel expenses as well as pay for lunch and evening meals. Accommodation will be arranged by the organisers

The organising committee: J. E. Celis, Chairman (Denmark), A. Grässmann (West Germany), M. C. Lechner (Portugal) and A. Loyter (Israel). 8469(D)

ANNOUNCEMENTS

JULY 16-20, I.C.R.F., D N A. Tumor Virus Meeting, Cambridge (I C R F, D N.A Tumor Virus Meeting Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, England). 8361(G)

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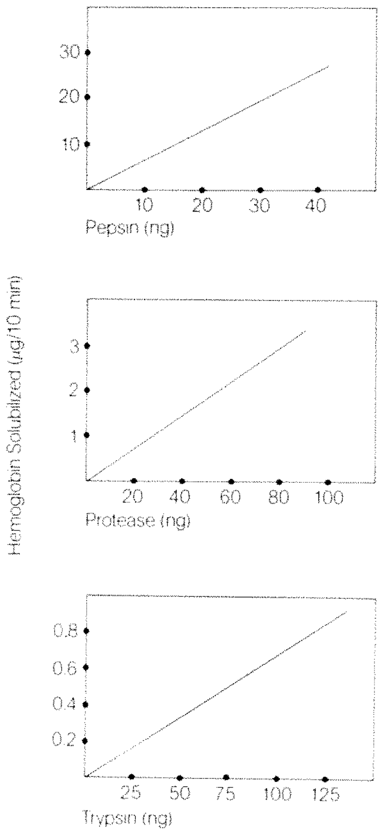

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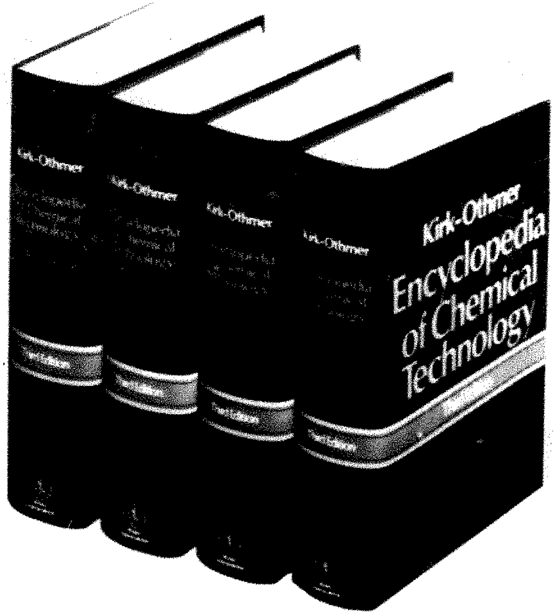

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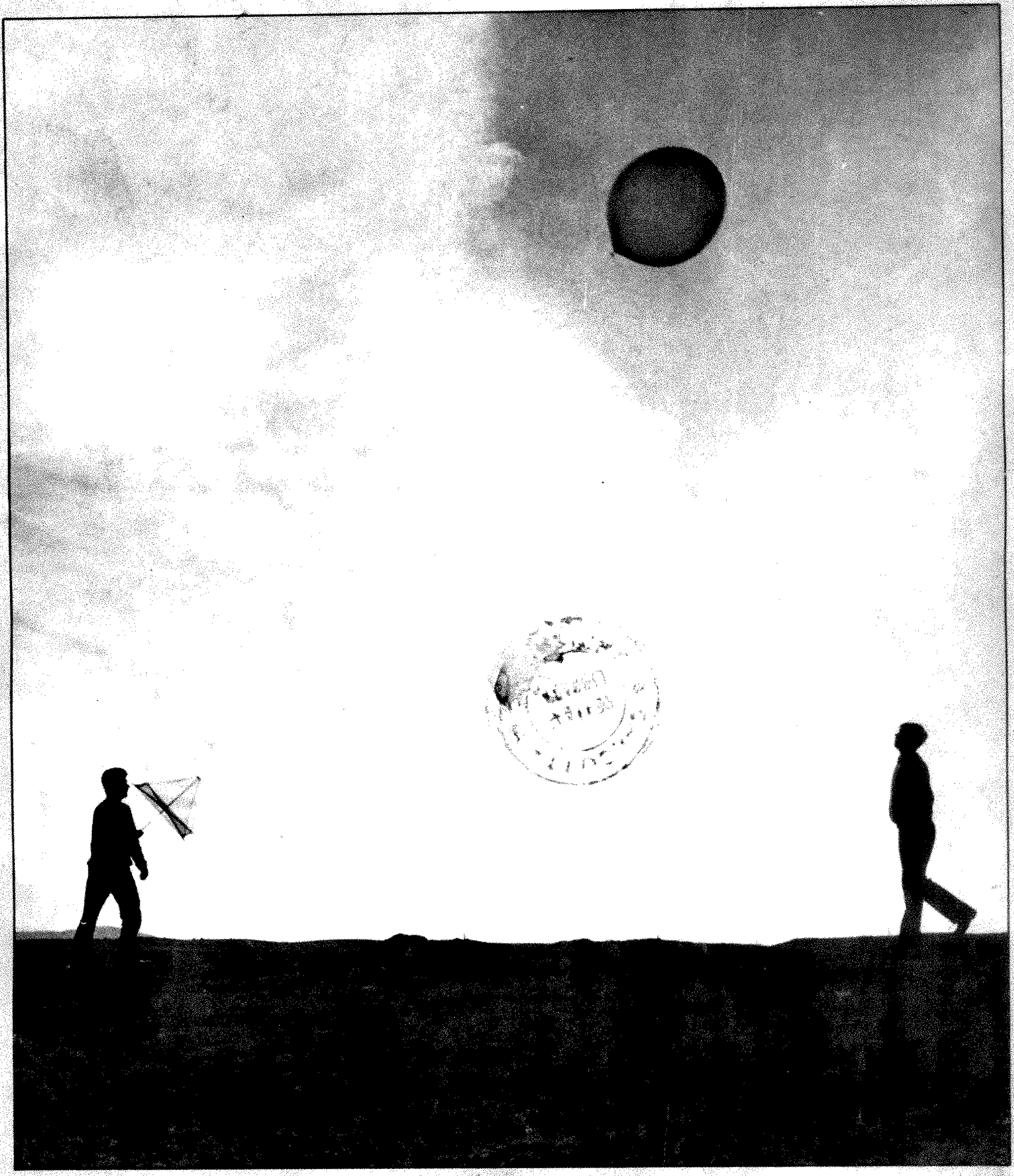

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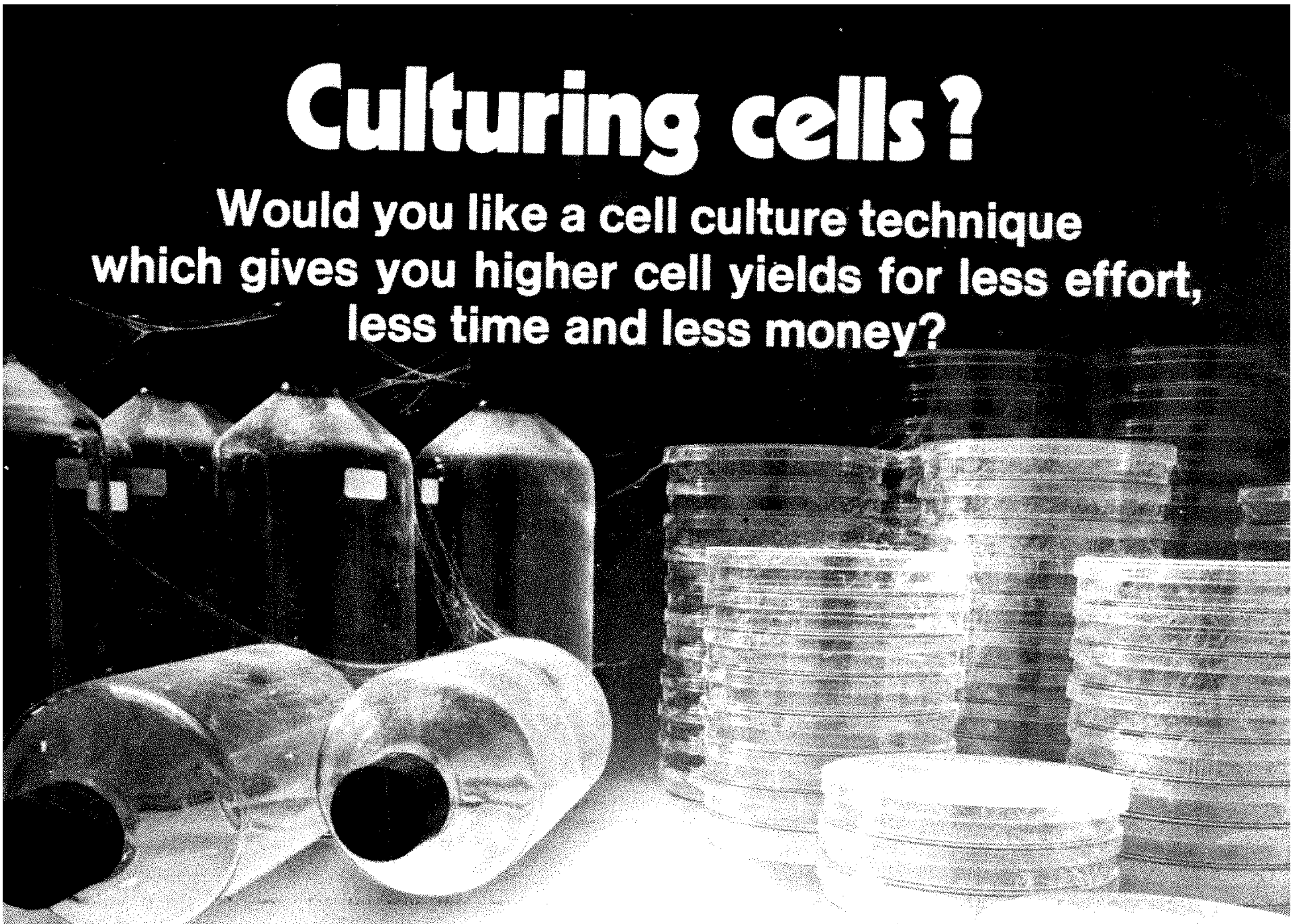

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Launching a wind-finding balloon, with a mesh radar reflector. A supplement on climatology starts on page 327 of this issue.
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nature 23 November 1978

Prejudicial to the interests of the state?

FOR nearly eighteen months the British courts have been occupied in one way or another with the cases of Crispin Aubrey, Duncan Campbell and John Berry, who had been accused of various breaches of the Official Secrets Act. Mr Berry is a former analyst in an overseas signals intelligence unit, and Messrs Aubrey and Campbell are journalists who recorded a three-hour conversation with Mr Berry on the subject of signals intelligence in February 1977. Immediately after the conversation all three were arrested.

A variety of charges were directed at the defendants, most notably under section 1 of the 1911 Official Secrets Act which prescribes up to 14 years in prison for those convicted of passing information for a "purpose prejudicial to the safety and interests of the state". Past prosecutions under this section have been confined to cases of espionage and sabotage; the purposes of the defendants seem to have been limited to the dissemination of information through journalism so a conviction would certainly have broken new ground in interpretation of the act. In the event, as proceedings wore on, with many peculiar asides down to the revelation that the foreman of one jury was himself a former soldier with the highly secretive Special Air Services Regiment, the Section 1 charges were dropped and the defendants were only accused under Section 2 of the act, which makes it an offence simply to transmit or receive any unauthorised information about what government employees do, see or make. Success for the prosecution under those circumstances was inevitable; there cannot be a single self-respecting journalist, nor for that matter government employee, who could not be prosecuted under Section 2 for some indiscretion or other. The judge, having earlier hinted that he did not intend to impose immediate prison sentences, lived up to his promise, sentencing Mr Berry only to a suspended six months in gaol and providing Messrs Aubrey and Campbell with conditional discharges.

It is important that this case be remembered for more than its elements of farce and anticlimax, and in particular a perspective has to be recognised on the wider issues involved. True, Mr Berry did pass on rather low-grade information concerning secret activities of the British government in the accumulation of intelligence—information which, it is claimed, is in any case almost in the public domain. And true there must be some small aspect of a government's activities which, for the sake of national security, is best not widely known (though Mr Berry's revelations do not seem, in any sense, to fall into such a category). But is it worth a quarter of a million pounds of public money to try and crush perpetrations of peccadilloes which could have been dealt with summarily in a magistrates' court? Maybe the judge's lenient sentences will have put heart into those who wish to reform our whole attitude to secrecy in government—a long-delayed activity. More likely the heavyweight support the government has shown for the maintenance of a *cordon sanitaire* around its activities will encourage those in government service who hold the view that nobody outside a charmed circle should know anything at all.

The Official Secrets Act casts a very long shadow. The same act that says it is undesirable that the current whereabouts of Britain's nuclear submarines should be widely known also says that any difference of opinion within a government agency is not for outside consumption. And the penumbra is even larger; those within the confines of the Officials Secrets Act are expected to develop a profound group loyalty which regards even such a harmless thing as a joking reference in print to the organisation as meriting official displeasure.

The results of decades of obsessive British secrecy and the harbouring of inside knowledge are around for all to see. In the Western world there cannot be a single government less open in its intentions with the public, and more distrustful of those, like journalists, whose profession is not just to repeat the official line but to try and shed light on the inner processes of decision-making. And the lack of easy flow of qualified people into and out of government service just reinforces the insider mentality. It is a total change of attitude, not a rewriting of the Official Secrets Act, that is needed to open up government—and this may take a generation. But by its vigorous pursuit of minor journalistic escapades the British government has shown it doesn't yet recognise the need for change at all. □

Inflation–and Congress–kill growth in funds for basic science

President Carter has had difficulty persuading Congress to increase support for basic research. Next year promises to be even harder. **David Dickson** reports.

Now that the dust is beginning to settle, it appears that apart from biomedical research President Carter has failed to deliver one of the promises made in his budget request to Congress for the financial year 1979, namely a "real growth" in funds for basic science.

The President had asked for a total of $3.6 billion, which would have resulted in an overall funding level 5 per cent above inflation. But the request has fallen victim to two factors: a higher-than-expected inflation rate, and a Congress reacting strongly to grass-roots demands for general reductions in federal spending.

Both factors continue to dominate planning for the 1980 budget, which will be announced in January. And despite indications from the president that he wants to see basic research protected from the most severe cuts, few expect that it will receive any particular privileges, or escape from the general constraints being imposed.

Speaking at a news conference earlier this month, for example, the President merely said that: "I have directed in the preparation of the 1980 fiscal year budget that basic research and research and development in general *should not be reduced as a percentage of the total federal budget*" (emphasis added).

The precise effects of Congressional actions on the science budget over the past year have yet to be officially calculated. The one bright spot—which tends to make the overall picture appear better than it really is—is a 34 per cent increase in the funds available for basic biomedical research through the National Institutes of Health.

Apart from this, officials of the American Association for the Advancement of Science have calculated the funds for basic science in all other fields will be 9.3 per cent higher than 1978. Dr Richard Atkinson, director of the National Science Foundation, gave a figure last week of 8.3 per cent; and others expect the figure to be even lower—close to the inflation rate.

The result has been a major disappointment to the scientific community. "The President's request for real growth in the science budget was met with great delight by the academic community; we are obviously disappointed by the lack of significant real growth in the budget", Dr Gerald Lieberman, vice provost and dean of research at Stanford said last week.

Overall, funds for research and development have not done too badly. The President asked Congress for an increase in R&D expenditure of 6.8 per cent, to a total of $29.3 billion. This figure was increased by Congress by a further $315.5 million, the major growth occurring in the Department of Energy, and the Department of Health, Education and Welfare (which funds NIH).

Furthermore, not all requests for increased support for basic science were handled roughly by Congress. There was general support for new research in the energy sciences, for example. Many Congressmen have been impressed by the performance of Dr John Deutch as director of the Office of Energy Research.

Similarly the budget for the National Aeronautics and Space Administration emerged relatively unscathed. The two main casualties were the proposed search for extraterrestrial intelligence, and continuing research on lunar samples brought back by the Apollo missions; however major new starts, such as the solar–polar satellite and the earth radiation budget satellite, remained intact.

The two agencies to suffer most were the National Science Foundation and the Department of Defense. The former had been slated by President Carter for an increase in basic research funding of 9.7 per cent; it ended up with an increase estimated by NSF director Richard Atkinson as 5.6 per cent, well below the expected level of inflation (although still, according to NSF officials, better than some had feared.)

Finally Congress responded unsympathetically to administration requests for an increase in expenditure for military-related research. The Senate, for example, although indicating that it might be prepared to consider increases in existing university-based programmes, rejected a proposal to start a new "Defense Science and Engineering Programme" for university research which would have been allocated $9 million in 1979 and $27 million in FY 1980.

The administration had been hoping that some of the research items cut from the DoD budget could be included in a supplemental request now being prepared following a presidential veto of the final defense appropriations bill, which included provisions for building an extra aircraft carrier unwanted by President Carter. However both Senate and House committees are reported to be unsympathetic towards such a move, arguing that such requests should be placed in the 1980—rather than the 1979—budget.

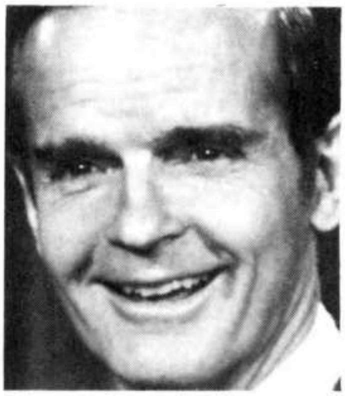
The net result of the various Congressional actions is what Dr Norman Hackerman, chairman of the National Science Board, has described as a "letdown feeling" among members of the scientific community.

Basic research, by agency (**$millions**)

	1979 Budget	Congress Approved	Change	% From Budget	% From 1978
NIH	856.0	1,019.0	+163.0	+19.0	+33.6
Other HEW	133.1	123.5	− 9.6	− 7.2	+28.1
NSF	754.9	741.9	− 13.0	− 1.7	+ 7.8
NASA	519.8	513.1	− 6.7	− 1.3	+ 9.6
Energy	467.5	465.5	− 2.0	− 0.4	+ 7.6
Defence	363.9	354.7	− 9.2	− 2.5	+10.8
Agriculture	251.9	256.2	+ 4.3	+ 1.7	+ 9.0
Interior	164.3	164.3	—	—	+ 4.6
Commerce	31.7	30.4	− 1.3	− 4.1	+10.9
Smithsonian	33.0	33.0	—	—	+ 5.8
EPA	30.0	24.0	− 6.0	−20.0	+14.2
Others	30.6	30.6	—	—	+10.1
Total basic research	$3,636.7	$3,756.2	$+119.5	+3.3%	+15.0%

From the AAAS report "Congressional Action on R&D in the FY 1979 budget".

●**Representative Whitten: "no friend of basic science"**



●**Senator Proxmire: "scourge of the scientific community"**

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As far as next year is concerned, things look little brighter. The President has repeatedly stressed the importance he attaches to supporting basic science to guarantee the long-term health of the country. In his nation-wide anti-inflation speech of 24 October, for example, he said that despite other budgetary constraints, "federal support for research and development will continue to increase, especially for basic research".

Some observers in Washington feel that the budget request for 1980 will propose a growth rate for basic science of 2 to 3 per cent above the expected inflation level. They take comfort, for example, from the fact that one of the first visits paid by Mr Alfred Kahn, the President's chief inflation fighter, was on Dr Frank Press, director of the OSTP, to confirm that he agreed with Dr Press' emphasis on the importance of research and development for long-term national prosperity.

The great unknown is Congress. This month's elections sent a clear message to Washington: US electors are demanding greater prudence in the way that the federal government spends its money and popular support gathers rapidly for those seen willing to yield a budget-cutting knife

As far as authorisation bills are concerned (those that represent policy decisions about the future directions of science) there is not likely to be too much problem. The new chairman of the House Science and Technology Committee will probably be Representative Don Fuqua of Florida, who has worked closely with agencies such as NASA in the past (and has several major research universities in his state).

Similarly the fortunes of science authorisation in the Senate are likely to remain in the relatively sympathetic hands of Senators Edward Kennedy and Adlai Stevenson Jr.

Appropriations committees, however—those that agree to disburse the funds—are a different story. In the House, the retirement of Representative George Mahon as chairman of the appropriations committee means that the position may be taken by Representative Jamie Whitten of Mississippi, the next in line on grounds of seniority

Mr Whitten is no great friend of the basic science community, or of its peer review system. This year the appropriations subcommittee of which he was chairman rejected an administration request for $30 million for a competitive grants programme for agricultural research in the 1979 budget, an attempt to shift away from the present system by which research funds are handed out to state land-grant colleges on a pro rata basis.

Defending this cut—half of which was later restored in the conference with the Senate—Mr Whitten said: "Congress must not allow itself to be placed in the position of being held accountable to the people for their research priorities established by a non-elected bureaucrat issuing grants to his fellow scientists". And his subcommittee's report contained detailed directions on how agricultural research money should be allocated

It is the Senate appropriations committee, however, which is giving the scientific community the greatest cause for concern, in particular over the implications of the loss of Republican Senator Edward Brooke

The chairman of the subcommittee responsible for passing both NSF and NASA funds is Mr William Proxmire, scourge of the scientific community with his infamous "Golden Fleece" Award for esoteric sounding research. (It was Senator Proxmire, for example, who was responsible for deleting most of the lunar sample analysis from NASA's 1979 budget).

Proxmire strengthened

The results of the elections have strengthened Mr Proxmire's hand. Last week he said "The fiscal scalpels must be applied to both military and civilian programmes, foreign and domestic spending, and to the sacred cows of the powerful economic interests . . No program, no interest group, and no region of the country, should remain immune to tough, detailed, and in depth budget cuts."

Up to now, Mr Proxmire's attempts to restrict funding for basic research has encountered strong—and frequently successful—opposition both from Mr Brooke, and from the ranking Republican member of the sub-committee, Senator Charles Mathias of Maryland.

However following Senator Brooke's departure, Senator Mathias is reported to be considering moving to a different subcommittee, and the concern of scientists that such a move could leave both NSF and NASA highly vulnerable has been heightened by the news that the subcommittee's minority staff member and aide to Senator Mathias, Mr Bob Clarke, is leaving the Senate to enter private law practice.

Much, of course, remains unknown about the future membership of committees, and will not be definitely resolved until Congress reconvenes in January. However one of the lessons brought home by the last Congress was the increasingly significant role played by intensive lobbying efforts.

Part of the substantial increase in support for basic biomedical sciences seems to have been due to a widely-publicised visit to Washington at the beginning of the year by a group of eminent biomedical scientists. In both public hearings and private meetings with Congressmen and their staffs, members of the group, which included three Nobel laureates and the heads of some of the leading US research institutions, argued that the long-term understanding of disease required a shift from a "disease of the month" mentality to a greater concern for basic science problems.

These arguments were subsequently reflected in Congressional action on the NIH budget Funding for cancer research, traditionally a recipient of Congressional largesse, was increased by 6.8 per cent; in contrast, the research budget of the National Institute for General Medical Sciences was raised by 25 per cent, to a total of $231 million Particularly significant was the emphasis placed in Congressional reports on the need to support "investigator initiated" projects, a point repeatedly stressed by the visitors to Washington, and the increase in funding for the Biomedical Research Support Grants programme.

A further illustration of the value of direct lobbying was memorandum sent by President Carter in June to the heads of appropriations committees and subcommittees requesting that they respect the integrity of the R & D package in his budget request Although the success of the memorandum was limited, some feel that it helped limit damage to the budget of, for example, the NSF

One message seems clear. Despite widespread awareness of the economic importance of research and development, the scientific community no longer enjoys any privileged position with respect to members of Congress, but must argue with everyone else for a share of the cake. "The universities, scientific societies and even industrial organisations will try to do even more than this year to increase support for research", Dr Jack Crowley, of the Association of American Universities, said last week

It remains to be seen whether President Carter's concern for the health of basic science is any more successful next year than this. Certainly his public statements, and the close relationship which his scientific advisers have been able to build up with the Office of Management and Budget, have reassured the scientific community that, unlike some of his recent predecessors, they now have an ally in the White House.

But since much of the science budget is in the form of nonmandated expenditure (unlike, for example, social security and Medicare), it remains highly vulnerable to marauding budget cutters. And it will take more than fine words and noble sentiments to secure any real growth for basic science in the immediate future. ☐

Post-docs take up ARMS

Short term research contracts are leading scientists to rebel, writes **Joe Schwartz**

THE organisation of scientific research in the UK has evolved to the point where there are now 10,000 people employed to do research on short term contracts and an additional 1,800 PhDs registered as unemployed with the Department of Employment. Combine these figures with recent cuts in public spending and the result is the inevitable development of labour-management conflict in an area of the economy that has always considered itself immune from such pressures. And, wherever a management view in defence of short term contracts is articulated (contracts are for specific pieces of work, the scientific mind burns out early in life, unfruitful research lines can be easily terminated), there is a labour point of view articulated by short term contract workers themselves.

ARMS, the Association of Researchers in Medical Science, is one organisation that is attempting to speak for the needs of full time researchers employed in the nation's hospitals and medical schools on short term contracts.

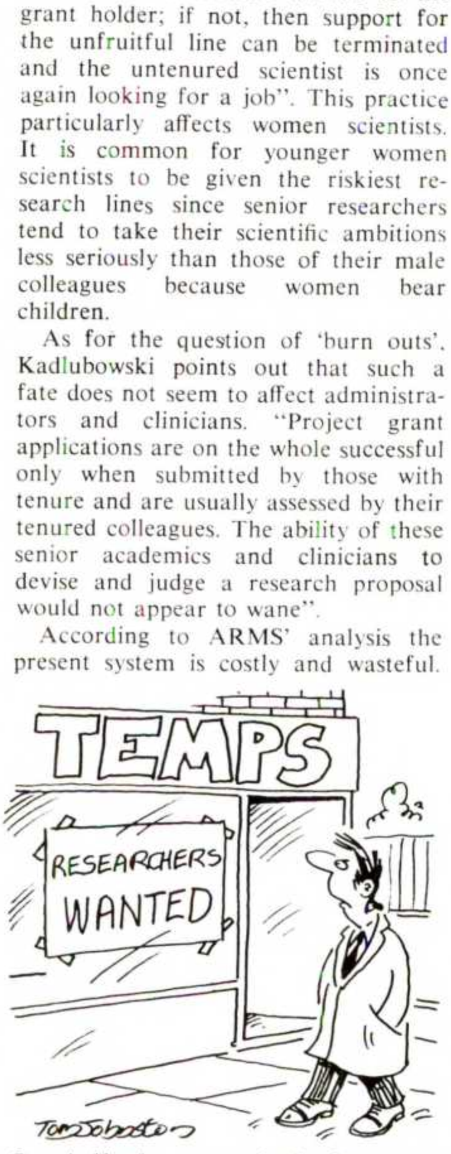
Based at Guys Hospital in London, ARMS was formed after a series of public meetings in July 1978. Its principal goal is to establish a career structure for full time non-teaching researchers analogous to university lectureship scales. Although not ruling out trade union affiliation, the organisation at present intends to negotiate with employers and to do educational work in an effort to change the present arrangements.

Membership in the organisation received a boost in September when the University of London which administers Guys instituted waiver clauses in short term contracts. Seeking to prevent 'onerous benefactions' to the research staff, the university demanded that researchers relinquish their rights to redundancy pay and claims against unfair dismissal. The outcry against this particular action has cast the university into the role of a 'bad' employer and could be rescinded if the researchers are able to maintain an active resistance to the measure. Brian Davis, Labour MP for Enfield and a member of the MRC, acknowledges that the MRC would like 'maximum flexibility' in its hiring and firing but is working to abolish the waiver clause as soon as possible. The Cancer Research Campaign, located at Mt Vernon Hospital, has removed the waiver clause after protests from the researchers there.

The waiver clause, however, is only the most recent grievance. Dr Mick Kadlubowski, Information Officer of ARMS, regards administrators' arguments in favour of contract work as specious and self serving. "The system prevents the untenured full-time researcher from having an effective say in research policy. Should a project be successful the honours accrue to the grant holder; if not, then support for the unfruitful line can be terminated and the untenured scientist is once again looking for a job". This practice particularly affects women scientists. It is common for younger women scientists to be given the riskiest research lines since senior researchers tend to take their scientific ambitions less seriously than those of their male colleagues because women bear children.

As for the question of 'burn outs', Kadlubowski points out that such a fate does not seem to affect administrators and clinicians. "Project grant applications are on the whole successful only when submitted by those with tenure and are usually assessed by their tenured colleagues. The ability of these senior academics and clinicians to devise and judge a research proposal would not appear to wane".

According to ARMS' analysis the present system is costly and wasteful.



One half of a researcher's time on a short term contract is spent settling into the project and then towards the end looking for a new job. After two or three contracts, the researcher is 30–35 years old and the contractors begin to find the price of experience too great. The experienced researcher is told to seek employment elsewhere and the funds thus 'saved' are used to boost the intake of juniors. "How can science progress when hard-won experience is so callously thrown away", asks Kadlubowski.

Davison of ASTMS; Akker of AUT.

ARMS has approached the DES and the MRC with little effect. Dr Anne Simmonds, chairperson of ARMS, is scathing about a recent MRC grant scheme: five-year renewable grants to age 35, grants to permit tenured academics to do full time research for a short time, and external full time research professorships for the clinically qualified. "At this critical time with their scant funds and these alarming unemployment statistics," she says "the MRC comes up with funds to allow the tenured time off to dabble in full time research, the clinician the opportunity to return to the field and progress without restraint to the professorial level, and another ten years of insecurity offered to the non-medically qualified."

The action of the MRC in totally ignoring the most urgent problems, the scandalous plight of full-time non-medically qualified researchers despite representations from clinical professors, deans of medical schools and the scientists themselves is utterly reprehensible".

Anne Simmonds is equally critical of university administrators. "Instead of raising their voices in support of some urgent action to resolve the situation they are aggravating it by looking for loopholes in the law to enable them to escape any commitment to their full-time researchers whatsoever."

Faced with this response from employers ARMS members have contacted ASTMS and the AUT, the two trade unions involved. "ASTMS calls us elitist and they want money on the line before they'll do anything", say members of the executive committee. Stan Davison, assistant general secretary of the 460,000-member ASTMS, denies this charge but admits that the problems of organising short term contract workers are formidable; the workforce is spread out over the entire country in small pockets of twos and threes; many scientific workers are indifferent or even hostile to trade unions; and research workers tend to be inexperienced and sympathetic to management arguments.

ASTMS is opposed to short term contracts and the unregulated em-

Dr Joseph Schwartz is on leave from City University, New York.

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ployer-employee relationship that currently prevails. Philip Hounsell, division organiser for ASTMS at Guys Hospital welcomes an organisation like ARMS. Hounsell feels that only by rank and file organising at the local level can sufficient strength be built to have a real effect. Both Davison and Hounsell emphasise however that without proper trade union organisation and support it is doubtful whether the authorities can be persuaded to act.

The 30,000 member AUT is approaching the problem at a higher level. It has negotiating rights for the universities and successfully negotiated national salary scales for researchers in 1975. John Akker, deputy general secretary, says that the AUT handles "scores of cases" of senior research workers, many with international reputations who have been made redundant at the age 40–45. The hardship is even worse he says because "many people are so specialised as to be unemployable".

The AUT offers legal services to members faced with dismissal and has been able to negotiate cash settlements in many cases and is in the process of formulating a code of practice for the employment of research staff. The AUT is attempting to move its efforts into the ministerial arena. "The MRC claims that the universities are the employer and the universities say since the MRC provides the money it is up to the MRC to sort it out", says Akker. Even if the research councils were to become actively involved there are still 78 autonomous institutions to deal with. The AUT wants the government to convene a committee with trade unions, universities, research councils and government representatives on it.

Whether the government is prepared to take on the issue is unclear. Certainly the universities are acting as if they have just discovered 19th century labour relations. Insisting on the value of piece work places academia in the league of garment manufacturers in a pre-trade union situation. This sensibility is reproduced in many researchers themselves who accept the line that a good student can always find a position and who reject trade union protection as being unprofessional.

But since research is an integral part of a modern economy some pressure exists for researchers to be able to make some changes. Certainly young potential scientists will begin to reject a scientific career if they become aware of the true conditions of research work. And there is nothing to prevent out of work scientists from organising just such a campaign if they are angry enough. ☐

People before pounds, say SRC

BRITAIN'S Science Research Council is no longer so worried about money, but is finding itself with another problem: manpower. The chairman of the SRC, Professor Geoffrey Allen, said last week that "the manpower problem is preventing us from diversifying".

Professor Allen was presenting a fairly encouraging annual report for the year to April 1978. The budget reduction of 1.7% per year imposed on the SRC by the Advisory Board for the Research Councils had been held to 1%, and merely another £20 million over the next 4-5 years would make the SRC comfortable. A 2-3% increase in the 'science vote' (the money allocated to the ABRC by the government for disbursement to the research councils) would give the ABRC all that the ABRC thinks is needed nationally.

Professor Allen is taking the training function of the SRC very seriously, and hopes to introduce courses for "topping up" in new technologies. This is "the major issue now" in training. And he wants to introduce a five person "think tank" into the Council which will concern itself with improving the links between university and industry. This call for five new people underlines Professor Allen's concern for SRC manpower.

The development of new activities at the Rutherford and Daresbury laboratories, whose subnuclear physics accelerators were closed in the year under review, is also raising manpower problems. The SRC, like many other government bodies, is under a directive to reduce its manpower —in the SRC's case at 1% per annum. This would mean a loss of some 30 people per year; but with fairly young staff it is a hard target to reach through natural wastage and early retirement.

While it has been possible to retrain many of the displaced high energy physicists at Rutherford and Daresbury in subjects like energy research and computing, it is not so easy to turn a physicist into a biologist to run, say, a biology support team at the new synchrotron radiation source. The central problem is that a few key people are needed, with special talents, to implement the changes which Professor Allen would like the SRC to undergo. "We now need different types of scientists to maintain our central facilities and links with universities", says Professor Allen. The SRC is a potential employer of scientists. But at the moment it is being held back.

Robert Walgate

Skylark rockets could be made from spare parts say UK geophysicists

A GROUP of UK geophysicists has come up with an idea which, in the early 1980s, could help soften the blow on auroral physics of the Science Research Council's (SRC) cutback in 'big science'. It has suggested that component parts left over from the SRC's abandoned Skylark rocket programme could be used to build as many as twelve extra Skylark rockets.

The decision to abandon the Skylark rocket programme was taken in 1975-6 as part of the SRC's plan to economise on 'big science'. Since then the British Aircraft Corporation, the rockets' manufacturer, has been slowly winding down production. Only one rocket now remains to be launched from Woomera, Australia, next May. During the 21 years of the programme, more than 250 Skylark rockets have been successfully launched.

The UK rocket programme now depends on two small rockets, the 7.5 inch-diameter Petrel rocket capable of launching 14 kg to a height of 200 km and the 10 inch diameter Fulmar rocket capable of launching 50 kg to more than 250 km. These are used for studying the lower and mid-ionosphere.

The 17 inch-diameter Skylark rocket is capable of carrying about 150 kg to altitudes as high as 750 km. It is the only UK sounding rocket developed so far which is capable of investigating the structure of the upper ionosphere and the origin of auroral particles. Although a Fulmar might have the ability to fly high enough, it does not have the capacity to carry the instrumentation needed to measure energetic incoming particles and electric fields.

The geophysicists claim that twelve Skylarks of varying size could be assembled, furnished with payloads and launched at no extra cost to the SRC's planned rocket programme over the next ten years if they were launched in fours at three-year intervals. This would mean launching fewer Petrels and Fulmars; but the value of the Skylark is such that it is thought there will be little opposition. Just how much support the idea could gain is what the group of geophysicists is trying to find out at the moment. By late next Spring it hopes to have sufficient knowledge of the interest and the kinds of experiments which could be flown to put forward a concrete proposal to the SRC for funding.

Judy Redfearn

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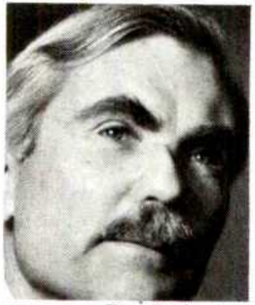
- **Nuclear waste disposal:** Highly radioactive waste from Britain's nuclear reactors could be safely stored underground by the 1990s, if suitable geological sites could be found, according to Dr Lewis Roberts (pictured below), director of Harwell research laboratory. In a lecture to the British Nuclear Society recently, he reviewed the present policy of the UK Atomic Energy Authority on radioactive waste disposal.

The means of solidifying high level wastes produced by the reprocessing of nuclear fuels, and the technology for building repositories, should soon be available, he said. At the moment, waste was stored as a highly radioactive acid solution in large tanks at Windscale. However, a solid was easier to contain and control than a corrosive liquid, so a technique for converting waste into a solid glass composition was developed at Harwell. This "vitrified" waste could then be placed in an underground store fitted with a simple cooling system and left, with minimum human surveillance, for the 500 years it takes for the highly radioactive actinides to decay.



An international programme, backed by the EEC, was underway to assess different sites for final storage, Dr Roberts said. Germany, Italy, Holland and Belgium were investigating the potential of salt and clay deposits, while Britain and France looked at hard crystalline rocks such as granite.

British exploration were running into trouble because some planning authorities (for example, in south-west Scotland and Northumberland) refused to allow the UKAEA to drill test holes. More research was needed to determine how safe the repositories would be, and the behaviour of containers and glasses under the conditions which might occur in storage sites. Despite these set-backs, Dr Roberts believed that research into final storage of radioactive waste should continue. The UKAEA was also looking into alternative storage sites, such as the oceans.

- **MPs seek comments on genetic engineering:** The social and political implications of the use of techniques of genetic manipulation, both in research laboratories and for industrial processes, are to be investigated in the UK by the Genetic Engineering Sub-Committee, which was set up by the House of Commons Select Committee on Science and Technology during the last session of Parliament.

The committee is asking any interested people or groups to submit written reports, even if they have not been specifically invited. The committee has also approached Government departments and agencies, professional bodies, companies and trade unions for reports on a wide variety of technical, commercial and ethical considerations. Submissions should be made before the end of the year, and the committee expects to report some time in 1979.

- **Chemical company claims dioxins a natural by-product of combustion:** The Dow Chemical Company, defending itself against charges that traces of chlorinated dioxins—one of the most poisonous of industrial chemicals—found in fish in the Tittabawasee River in Michigan had leaked from the company's pesticide plant, claim that its scientists have discovered the compounds to be natural by-products of combustion.

In what is said to be a "significant breakthrough" in the chemistry of fire, the company scientists say that small quantities of chlorinated dioxins, well below the level that poses a hazard to human health, have been discovered in coal and oil-burning power plants, in home fireplaces and in charcoal grills. "We now think dioxins have been with us since the advent of fire. The only thing that's different is our new-found ability to detect them", Dr Robert Bumb, research director of Dow's Michigan division, said last week.

The eating of fish from several rivers near the plant was banned by state and federal environmental agencies earlier this year following the discovery of traces of chlorinated dioxins in both soil samples and game fish.

- **Petrol additive found to be potent carcinogen:** A chemical widely used as a petrol additive and a fumigant for agricultural crops has been found to cause a "high incidence" of cancer in laboratory animals by the National Cancer Institute. According to the institute, tests have shown ethylene dibromide (also known as EDB or 1,2-dibromoethane) to be one of the most potent animal carcinogens ever found, and must be considered capable of causing cancer in humans.

The National Institute for Occupational Safety and Health has estimated that in 1977 about 9,000 workers were exposed to EDB in industrial settings, and that a further 650,000 service station attendants were exposed to much lower levels of EDB. The chemical is added to tetra-alkyl anti-knock fluids to remove lead from the engine's combustion chamber.

In a two-year study, during which large doses of EDB were administered to rats and mice, stomach cancers developed in 58% to 90% of the rats and 56% to 94% of the mice, depending on the sex of the animal and the dose received. Some cancers developed less than two months after the beginning of the experiment, and the death rate among experimental animals was so high that the experiments were discontinued when the programme was only half-way through.

- **World population growth is slowing:** The rate at which the world population is growing has begun to slow down. Since the 1960s, birthrates have fallen by 15% in about half of the countries of the developing world, according to Rafael Salas, executive director of the UN Fund for Population Activities. However, Salas adds: "It would be unwise to conclude that the population problems of individual nations as a whole are at an end, or anywhere near it."

Another big world population rise is forecast, since at least 40% of the people of the Third World are under 15 and approaching child bearing age. In his annual report, Salas puts the lowest estimate for world population in the year 2,000 at 5.8 billion compared with today's total of about 4 billions. In poorer countries the age structure is changing towards a higher proportion of very young and very old people. The trend is causing concern at the UN, because it will put strain on economic and social resources for education and employment.

- **Naming the unifying gauge theories:** Some physicists are suggesting that the time has come to rename some of the new forces and interactions in particle physics. Greek, which was the basis for nomenclature until the arrival of modern idiosyncrasies such as "colour" and "flavour", could be used as the source for the new words, some physicists suggest.

The theoretical developments which appear to have unified the weak and electromagnetic interactions failed to

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provide a new word that physicists could use to describe the forces, apart from the indirect term the "Weinberg-Salam model" and the unimaginative "electro-weak". A candidate for the title is "asthenic force", originally suggested by Chanowitz and Ellis and now revived in the October edition of the CERN Courier. *Asthenis* is a Greek adjective meaning "not strong", and so the word has the advantage of covering all phenomena not associated with the strong force. Chanowitz and Ellis suggested that the unified gauge theory of weak and electromagnetic interactions could be described as "quantum asthenodynamics". Pursuing their Greek source further, they proposed that the grand unified gauge theory of all the forces could be named "quantum holodynamics".

- **Lasers:** The laser light show in London's Oxford Street, the first of its kind to be used in Christmas decorations, was switched on last week, despite a safety inquiry which nearly banned the show. The six krypton and argon lasers have splitters to produce red and green beams of light. The system was originally built to sweep the laser beams along Oxford Street 20 feet above the ground. But the risk of a beam being reflected by a window into someone's eyes forced the Health and Safety Executive and Westminster Council to insist on the lasers being fixed in one position. The beams would be allowed to move only if every window along the route was blacked out.

'Problem' solving still claims Poland's scientists

"DESPITE many shortcomings which still exist, we have the right to state that our science is carrying out its tasks more successfully each year". So claimed Piotr Jaroszewicz, Prime Minister of Poland, speaking at the opening session of the Sejm (Parliament) last month.

For a head of state to present the Government's programme on science policy is somewhat unusual. Partly it may be attributed to what Jaroszewicz called the "particularly active part" which science must play in overcoming "the difficulties experienced by our economy at present" (disastrous results in agriculture for the fifth year running, difficulties in earning the hard currency needed for vital raw materials and over-consumption of labour-time, energy and raw materials).

Moreover, in September, Poland's trouble-shooting Minister of Science and Higher Education, Sylwester Kaliski, died after a car crash, and the presentation of the science programme by so eminent a figure as Jaroszewicz seems a further assurance of government commitment to the policy so largely associated with Kaliski's name, although originally worked out by the Polish Academy of Sciences —a structured hierarchy of R and D "problems".

In this scheme, problems vital to the economy range from "State problems", through "Key", "Interdepartmental" and "Departmental" down to "Branch" problems. The differences are both administrative (who is responsible for funding and coordination) and a matter of actual priority.

There are seven "State" problems at present—copper, coal, building industry, food, electronics, cancer and water/environment, and it seems likely that, in the near future, energy and the chemical industry will receive the same status.

The urgency of the "state" problems is considerable. In the case of building, the construction of a new campus for Poznan University has been approved and introduced into the central plan of the Government, with allocation of 4,500 million zloty. Yet, according to the pro-rector, Dr S. Kozarski, the University is having considerable difficulties in getting the construction under way, simply because of the overall state of the building industry.

According to a spokesman for the Ministry of Higher Education and Science, the system of problems is "working, though not so well as we had hoped at first". During the next five-year plan, he said, there would be greater freedom of decision-making left to the researchers and fewer referrals to distant committees in Warsaw. At present, the heads of research teams are far to circumscribed by "reports and byelaws".

Does this direction from above restrict academic freedom? No, said the rector of the Poznan Polytechnic, Dr Boleslaw Wojciekowicz, where some 60% of research comes under the central scheme. There is no compulsion, he said, for any institution to accept any problem it does not feel it can tackle. "Freedom of science", the rector exclaimed, "includes the taking on or not taking on of government research".

The system does serve as an indicator to research institutions what is important now and in the future, and provides a method of concentrating both personnel and financial resources. Moreover, any institution—his own Polytechnic, for example, can at any time propose some new problem to be included in the scheme. At present, he added, his Polytechnic acted as research coordinator for three such problems, at the "Interdepartmental" level, coming therefore, under the joint authority of the Academy of Sciences and the Ministry.

There also remains the other 40% of research activity at Poznan outside the central scheme. Some of this includes direct contract work for industry, but funds are still available for basic research direct from the Ministry of Education, and are distributed to the individual Institutes concerned. The Directors of these institutes have a fairly free hand in allotting these funds, and can finance any project they consider promising.

So far, too, international cooperation does not seem limited to the formal Government policy of Comecon cooperation. According to the Ministry spokesman, since Comecon is primarily an organisation for economic matters, the intra-Comecon links in higher education are somewhat weak. When there is joint research, it is directed not through the Ministry of Education but through the specialised ministries which have their own research institutes at their disposal.

Dr Wojchiekowicz put it more bluntly. "If the ministry proposes a key problem to us, we are not interested in whether another country needs it as well—though of course if we know others are working on it, we naturally try to establish contacts". His Polytechnic, he said, had collaboration agreements with 26 foreign institutes—including some in West Germany, Holland, France, and the USA. "But with non-Comecon countries, the basic problem is the perennial one of currency."

It would be strange, indeed, if the precious funding for foreign cooperation did not go first and foremost to government proposed problems, and, indeed, a number of examples could be cited. There is close cooperation with the UK on safety in coal mines, for example (coal is a "State" problem), while the "urban forest" conservation scheme (a "Key" problem) benefits from Landsat photographs under a

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NASA–Polish agreement.

It would seem inevitable that this concentration on the coordinated problem concept should have its effect on the higher educational system generally. Jaroszewicz's policy speech proposed that "scientific and research topics of particular importance of the economy should be stressed even more not only at the research stage but in higher education also."

Likewise, the Ministry of Education spokesman indicated that future plans include "establishing closer links during study with productive activity". He also said that serious consideration was being given to the possibility of "reattestation"—the granting of professional qualifications not for life but for a limited period only, with renewal depending on the candidate's career in the meantime

Not surprisingly, perhaps, a number of academics are privately concerned about the ultimate effect of the problem concept on Polish science. The freedom to accept or reject a government proposal is, they feel, a somewhat circumscribed one. Academic freedom, they point out, was one of the principal issues of protest against the proposed constitutional changes in 1975-76. Although the ensuing "debate" resulted in a certain toning down of the clauses relating to Party preeminence, there still seems to be considerable nostalgia in certain circles for the concept of autonomous universities and academies electing their own senior officials without outside influence, instead of requiring, as at present, government and Party approval. **Vera Rich**

Leather science as alternative technology

by Paul Calvert

who is on leave from the University of Sussex

In developed countries it is easy, as a scientist, to adopt the view that most research stands a chance of being applicable and most applications of science will lead to an improvement in our overall well being. This is much less true in developing countries; given their labour surplus and shortage of capital any technological advance which replaces men with machines may be bad An example of a research organisation trying to serve a traditional, cottage industry is the Central Leather Research Institute (CLRI) in Madras.

The CLRI was set up under CSIR in 1952 with the task of providing scientific support to an industry which consists mainly of small factories and individual, often illiterate, craftsmen spread throughout India. An additional, uniquely Indian, dimension to the problem is that most of the workers are members of the "untouchable" castes Until 1971 the director of the institute was Professor Y. Nayudamma who subsequently became director-general of CSIR.

Writing in 1967 (*Science and the Human Condition in India and Pakistan*, edited by Ward Morehouse, Rockerfeller University Press, 1968) Nayudamma described the philosophy and operation of the CLRI. Much of the institute's effort is put into extension centres close to concentrations of the leather industry and extension workers who can visit and live in the villages. These workers must respect the skill of the village craftsmen and must be able to talk to them at a level they can understand. Thus a method for controlling *p*H during tanning would be presented simply as a series of coloured packets to be added at different times in the process.

Other programmes include a "guest tanner" who can visit and work in the institute, and monthly visits by the director, with local officials in tow, to the industry in a particular state. Institute staff have also been sent in to revive businesses which are running at a loss, the idea being that this can be a powerful demonstration of the benefits of science. CLRI is closely associated with the University of Madras and runs BSc and PhD courses in leather science as well as refresher courses for teachers from regional tanning institutes.

Since going to CSIR, Nayudamma has been able to develop his ideas of the role of science in India and has propounded them in two recent lectures. In a paper called "Endogenous development· science and technology" given at a UNESCO meeting in Paris in April, he discussed science and technology in developing countries In his eyes development is not simply a question of economic growth but of development of a people in terms of dignity, resourcefulness and social justice. Such patterns for development cannot be imported since they must suit the society in question.

Nayudamma quotes Gandhi's vision of self reliant, egalitarian rural communities using labour intensive methods and local resources but intellectually open and active Science, based as it is on experiment and reason, should reduce the hold of superstition and increase rationality and enterprise

Nayudamma sees imported technology as imposing the values and culture of the developed countries on the less developed. The success with which Japan imported technology is due to the strength of their indigenous technology which enabled them to modify the imported methods to suit their own needs So some non-imported route to technical development is needed in order that a level is reached at which constructive use may be made of imported technology This "alternative technology" has as its goals to provide employment, meet basic human needs and build confidence and self-reliance It would include use of local resources, recycling, have a low energy demand and would be a weave of traditional and modern methods

At the moment manufacturers tend to prefer imported processes which come fully developed, with known brand names as compared with local inventions which are untried and involve more risk. To overcome this, processes are offered with the government providing 50% of the risk capital and guaranteeing the result.

In "Developing Science for Development" a lecture at IIT in Madras last year, Nayudamma outlined the kind of research organisation he saw as necessary to serve a developing country. The key to science within this framework is one of setting clear goals for researchers At present they tend to be judged only on their status in international science so there is little encouragement for working on strictly local problems, particularly for scientists trained abroad

Further, scientists must be in close contact with the rural population; it is important to know the people whose problems you are solving and these are not the westernised elite but the rural majority with more basic needs This lecture was given at a time when CSIR was under threat of being broken up and apportioned amongst the various ministries, a good example, perhaps, of imported ideas (see *Nature* **270**, 89; 1977). Nayudamma strongly defends the existing structure which survived, and calls for a clear lead from the government as to what technology is needed When clear objectives are defined scientists usually achieve them.

In helping the leather industry CLRI must also tend to come into conflict with the caste system which is deeply ingrained in rural society In recent years the untouchable castes including leather workers have tended to move from the villages into the cities where they have more political and social freedom In the case of leather workers this will include setting up small businesses or co-operatives By becoming involved with small scale industry,

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scientists thus also become very exposed to the ebb and flow of political forces.

Current fundamental research at CLRI, in addition to studies of tanning and degradation in leather, include extensive work on grafting synthetic polymers to collagen (*Makromol. Chem.* **175,** 729 (1974); *J. Polymer Sci.* **9,** 3199 (1971); *J. Appl. Polymer Sci.* **16,** 1975 (1972)) to produce wear resistance, water repellant and glossy surface finishes on leather. This sort of work is coming more into its own as India exports more finished as opposed to semi-finished leather. These treatments can be simply carried out and so are appropriate to small-scale industry. The institute also provides courses in design of leather goods so that the industry is able to keep up with fashions in world markets.

Thus CLRI provides an example of a scientific institute serving "alternative technology" and demonstrates the considerable effort that must be put into keeping communication open between the users and the scientists. Nayudamma, following his own precept that a director should return to the laboratory at the end of his term, is back at CLRI as a "distinguished scientist". He is studying the impact of CLRI on the leather industry over the last 25 years. The current director is M. Santappa who is also professor and head of the department of physical chemistry at the University of Madras. Santappa's main scientific interests before becoming completely involved at CLRI were in polymerisation of synthetic polymers.

The big question confronting this approach to science is whether a technically developing cottage industry could ever compete with heavily capitalised manufacturing industry. About 10 years ago much effort, particularly by Du Pont, was put into making artificial leathers consisting basically of a foam and skin structure of PVC. Had this succeeded, as it almost did, it would have drastically reduced the market for natural leather. In an industrialised society if workers lose their jobs under these circumstances they have a reasonable chance of getting others. Traditional craftsmen, such as Indian leather workers, do not have this flexibility, particularly if they are modernised to the extent that they depend on exported goods for their livelihood. □

Third World needs 'barefoot' science

How does a water tap work? **Michael Moravcsik** and **R. H. B. Exell** explain why knowing the answer can be a breakthrough for an African village.

The practical need for science in developing countries is rarely discussed. Debate usually rises to a general, analytical, or theoretical level. There is no question that such discussions help in understanding the role of science in those countries, and assist the proponents in convincing incredulous officials in national, international, and other decision making organisations, that science is necessary. And they also boost the morale of the scientists in the developing countries—who otherwise have many reasons for being discouraged and frustrated. But one should also be aware that the need for science can be much more down-to-earth.

In this brief note, therefore, we want to present a few examples where exposure to science as a way of thinking and as a method of inquiry and problem solving could play an important role in very simple everyday situations.

The examples are taken from "real life" situations that we have encountered during our stays in developing countries. Colleagues of ours could doubtless add dozens of such examples to the list.

Keeping cool: In urban buildings in tropical areas, built using modern techniques, the top floor is often unbearably hot. The reason is simple: the usually flat roof absorbs solar radiation, and no provisions are made to either insulate the roof from the ceiling of the top floor, or to design the building with a simple double roof with air space between the two for ventilation. Ironically, such features are often included in the traditional, older, and rural structures, but since the scientific reason for such features is not comprehended, builders cannot distinguish between those features of traditional architecture which are functional and those which are ceremonial or due to limitations of construction technology.

Keeping hot: In numerous developing countries tin-box ovens used on charcoal stoves are common. They simply consist of a usual thin-walled surplus tin-box, placed on top of a regular iron charcoal stove (similar to the hibachis popular in Western countries for use in backyard barbecues).

It does not take very much science to realize that the efficiency of such an oven, and also the attainable temperature inside, could be increased if all sides of the tin box except the one in contact with the stove were insulated. A little more science tells us that such a saving in fuel would be quite substantial, very rapidly returning the small initial investment in one of the available insulating materials.

Current problems: Electrical wiring in developing countries is often installed very flimsily and haphazardly, so that breaks in the wiring and other types of damage occur frequently. In such instances there are many accidents as well as further damage due to incorrect rewiring. For example, we have seen instances when the break in a two-strand wire was "repaired" by joining all four dangling wires in one knot.

Even the most qualitative and simplest *conceptual* understanding of current as a flow (and hence a schematic knowledge of what bears the unnecessarily forbidding title of Kirchhoff's laws) would do much toward preventing such occurrences.

Turning on: Recently, in one of the developing countries, the supply of tap water became quite intermittent for several months. It was then found that during those hours of the day when the water supply did not operate, much subsequent loss of water was initiated by taps being left in an "on" position during the time when the supply was off. People would try the tap, see that no water was coming, and then leave the tap without turning it to the "off" position. When the water supply re-

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turned, the water kept flowing sometimes during the whole day until somebody happened to return and turn it off.

Water being expensive, one of the important contributing factors in this apparent neglect is likely to be the complete absence of an image of a water flow with a tap being a gadget to transmit or obstruct this flow. When this simple point is not understood, there is no more "reason" for turning the tap to the "off" position when the water fails to emerge. This is science on such an elementary level that most of us might find it difficult even to realise that it is science.

Roofing in the tropics: applying simple scientific principles can make a hot house cool.

Counting squares: The next two examples are perhaps more mathematical than physical, but they again stress the importance of understanding concepts rather than following recipes.

A junior lecturer at a university, engaged in an "applied" problem in botany, was confronted with the task of measuring the surface area of leaves. He kept postponing the measurements, however, because an expensive planimeter ordered from abroad had not arrived and, according to some textbook, was *the* instrument to use for such measurements. He was amazed that tracing the leaf on to readily avail-when one of us pointed out to him able graph paper with a square millimetre network and then simply counting the squares within the traced figure yields the desired answer with as great (or greater) precision than would have been obtained with the planimeter.

Saving time: In mathematical calculations related to applied scientific or engineering problems, the lack of a conceptual understanding of mathematics often prevents the practitioners from using approximation methods (particularly numerical ones) which yield the answer to the required degree of precision much easier than fancy analytical methods or computer evaluations of the "exact" problem. When in a calculus of variations problem, the shape of a curve has to be optimised, the approximation of the smooth curve by a chain of straight-line segments can reduce the problem to the solution of a simple set of algebraic equations.

The same is true in complicated boundary value problems in differential equations of heat transfer and other problems. Countless hours of precious computer time could be saved on expensive and scarce electronic computers in developing countries if their users were better trained in the fundamental concepts of science and mathematics and relied less on recipies from enginering handbooks or on imitating calculations described in esoteric books dealing with different problems and different tolerance requirements.

Hot water: A solar water heater is to be designed. Even if the basic qualitative principles of such a design are understood, much will depend on the ability to carry out a fairly reliable estimate of heat losses, conversion efficiencies, and the like. For this the scientific principles underlying these phenomena must be clearly understood, because engineering handbooks or the lecture notes saved from university training will hardly give automatic recipes for such "non-standard" situations.

If an engineer claims that he could make steam with such a solar heater, he must also be able to estimate with sufficient accuracy the temperature and the amount of such steam. In the absence of such ability, his project remains an academic exercise and cannot be introduced to "real life" situations where the economic considerations are important and where a quantitative assessment is essential.

A similar situation exists when a claim is made for a design of a practical solar-heat dryer of agricultural products. In this case the useability of the resulting products as well as their quantity will depend crucially on the quantitative factors in the design which, as before, must be estimated on the basis of the scientific principles underlying the engineering applications.

So much for the examples. In seeing them, the reader may advance various objections, at least two of which should be briefly discussed.

The first objection may be that our examples depend not so much on the lack of science but on the lack of "common sense". It is relatively easy to dispose of this objection. Indeed, to a person imbued with some conceptual background in science and technology, the examples may appear a matter of common sense. This is so, however, because he has internalised simple concepts of science and mathematics to such an extent that viewing the world in that light now appears to him a matter of common sense and no longer a matter of science and mathematics.

This is the main point we wish to make: in developing countries such internalisation and fusion of scientific and mathematical conceptual elements with the customary empirical view of the world is very rare, whereas it is much more frequent (though far from universal) in the more advanced countries. This shaping of man's world view is, in fact, one of the primary goals of science in developing countries, and yet it is one that is much too infrequently included in the discussions that prevail throughout "development" literature.

The second objection may be that the missing elements in each of our examples could be provided by a well-trained technologist and engineer and hence science is not really directly involved. In some idealised world this statement may carry some validity. In practice, however, the technological and engineering education, particularly in the developing countries, is such that a functional understanding of the conceptual scientific basis of technology is extremely rare among the graduates of technological and engineering institutions. This is partly so due to directives based on a total misunderstanding of the nature of applied scientific research and of technological development.

According to such directives, engineers and technologists should be given a narrow education in which "unnecessary" abstractions are weeded out and training with respect to a very narrow set of specific projects is the goal. In part, however, the lack of conceptual understanding is due to the lack of contact of these students with teachers who are, themselves, involved in some scientific work and who therefore have had a personal experience with how science is actually done, as distinct from how it appears from the textbooks. Thus we conclude that we need a much greater attention to education.

correspondence

The Natural History Museum needs the best of both worlds

Sir,—The current controversy concerning the Natural History Museum's new exhibition scheme and philosophy, eptiomised in the opposing views expressed by Drs Miles and Halstead (26 October, page 683), highlights problems which certainly deserve the "fullest possible public discussion" as Halstead suggested. While my personal opinion is no more (or less) valuable than that of the next man, as the father of two of the museum's avid users, and also as an ex-curator of one of its scientific sections, I can, at least, see the situation from two different points of view and can make some observations rather more frankly now than when I was still a staff member.

The tremendous gulf between the expressed views suggests that the fundamental question of the museum's role remains to be resolved—despite two centuries of more or less continuous discussion. No-one would dispute the fact that the museum has two distinct general functions, both clearly identified, as Miles points out, by the British Museum Bill of 1753, and as valid today as they were then. The first of these, to carry out research into the natural world, occupies almost two-thirds of the museum's total complement of 750 and sails serenely on with hardly a ripple from the outside world to rock the scientific boat. It is the second, more public role, which attracts all the attention even though it is dealt with on only seven sides of the museum's most recent 170 page report to Parliament.

But while these general functions have remained unaltered, the techniques and approaches needed to discharge them have changed, and will continue to change, throughout the museum's existence. For just as it would be totally inappropriate for the present scientific staff to approach their work in the same manner as their Georgian predecessors, the public galleries should surely bear little resemblance to those of 200 years ago. For one thing, both in numbers and in social stratum, the public served then was very different from that using the museum today. In its early years no more than a few dozen privileged visitors could view the British Museum exhibits each day—and even then only by appointment with a Keeper; as a son of the eighteenth century equivalent of a lorry driver I would personally have had little chance of stepping through the door, let alone of becoming a Curator!

Second, the museum now operates in a different educational environment. The present-day flood of scientific information for the "layman" through books, films, radio and, above all, television did not exist ten, or even one, generations ago. No museum exhibit should attempt to compete with the slickness and immediacy of a television presentation, for whatever you may think of their particular contributions, a Bellamy or a Cousteau can reach a larger and more varied audience in one hour than the BM(NH) could hope to reach in ten years. Surely the museum should adopt a complementary role in these circumstances, concentrating its efforts on those things which it can do better than the mass media by exploiting its particular strengths, its in-house scientific know-how and its vast collections of material.

The official museum view of the way forward seems to be to attempt to put over concepts and ideas to a general public largely devoid of any background in natural history. The opposing "Halsteadian" view, which appears to have the support of a significant proportion of the museum's own scientific staff, seems to favour a return to a more traditional type of "scholarly" display with many examples of the animals, plants and minerals themselves from which the informed visitor can draw his own conclusions. Either approach alone would be dangerous, sterile and damaging to an institution for which so many of us feel the deepest affection. If Dr Miles really believes his survey results which told him that the old displays taught virtually nothing, he should have been with my wife and I when we took our 6-year-old son on his fifth or sixth visit to the museum some years ago. After a depressing appointment with a child psychiatrist had confirmed that he had severe learning difficulties, we were considerably encouraged to have him point out to us the differences between African and Indian elephants in the now dismantled Main Hall exhibit! Like most kids he still likes to see lots of dinosaurs, whales, fishes and so on, and much prefers them to the Hall of Human Biology, which, after its first month, was looking pretty dog-eared by the time we got to it. On the other hand, Dr Halstead's plea for "scholarship" seems equally pathetic. He must realise that not many of us would come up with an evolutionary theory from a perusal of a Cuverian arrangement of specimens, while I cannot imagine what diabolical deeds he envisages the "more inarticulate sections of the community" being incensed to perpetrate as a result of exposure to the Natural History Museum's "social engineering".

However, I feel much more sympathy for Halstead's main worry of a "serious breakdown of communication between the museum's Department of Public Services and the scientific staff . . .". It's all very well to cite the dismay of the museum's "world leading ichthyologist" and "dinosaur expert" at the loss of the exhibits of their respective groups, but they didn't reach their present eminent positions on the strength of their efforts in the exhibition sector. For like the rest of the scientific staff they know that their professional advancement has depended entirely on publication; time devoted to exhibition work has therefore tended to be spent grudgingly and, at least in the past, was often restricted to topping up a dried out bottle or replacing a lost label. Under such circumstances it is little wonder that a them-and-us situation developed between the public and private sections of the staff, with the scientists taking little or no interest in the exhibits for which they were nominally responsible. The scientists can't expect to have their cake and eat it, for if they want a real say in the fate of the exhibitions they must be prepared to be involved. In return, the museum establishment must demonstrate the value they attach to such involvement in terms of career structure and promotions. The Department of Public Services seems to have evolved to fill a gap resulting from the mutual distrust felt on precisely this point.

If there are now two quite distinct, but genuinely held, opinions of the type of exhibitions which the museum should mount in the future, it is surely not too late to exploit the best features of both views. The projected building programme within the museum's existing walls is supposed to provide four times the existing exhibition space over the next three decades. Such an expanse must be capable of housing the traditional scholarly exhibits favoured by Halstead, the exciting new conceptual approach advocated by Miles, and the fun exhibits which the dominant less than 11-year-olds can stare at in wonder and awe and remember in the recesses of their mind for the rest of their lives.

Yours faithfully,

A. L. Rice

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UK

New policies are correct

Sir,—There seem to be three separate strands in the recent controversy on the new display policy of the British Museum (Natural History): whether displays should be for scholars or for the general public whether they should be organised by research scientists or by exhibition staff, and whether the former balance of displays should be retained. In each case, I believe that the new policies of the BM(NH) are basically correct.

Until recently, the majority of the displays were little more than a collection of labelled specimens. There was little attempt to point out any features of particular interest, or to help the visitor to appreciate their significance to current scientific theory. Even those displays directed towards university students were suited to a level of knowledge that was not usually attained before the second year of a zoology degree. Similarly, those exhibits that were directed at the reasonably well-informed adult were accompanied by complex and lengthy labels, their comprehension requiring a considerable degree of dedication and time. Finally, many of the exhibits were so old-fashioned as to merit illustration in any book of the history of museum display technique. It can be no surprise that the majority of the visitors to the museum had, as Dr Miles stated "learnt practically nothing". It is surely appropriate that the museum should attempt to inform, as well as interest, the throng of children and of scientifically uneducated adults who nowadays pack its halls.

Should we really expect that the prime responsibility for the new exhibition approach should rest with the scientific staff, already committed to programmes of research and curation? It is no secret that, in any museum, many research workers have little interest in the problems of the design of displays, even if they are of completely conservative and scholarly type. It is even more unlikely that many of them would wish to learn and to apply the theory and techniques of modern displays directed at those with no scientific qualifications. (This is not to say that the scientific staff should have no role in this, and many of those at the BM(NH) certainly seem to feel that they have not had sufficient opportunity to comment on the new policies, or to ensure that the scientific theories illustrated are appropriate and not merely those currently in vogue).

The balance of exhibits is a complex matter. I feel that it is only appropriate that the museum should attempt to let visitors understand themselves as human examples of the world of nature, and to educate them in the basic processes of ecology. (Not even the museum display staff would pretend that every exhibit in the Hall of Human Biology is an unqualified success, but even the BM(NH) must surely be allowed to make the occasional mistake). Furthermore, there can be no doubt that invertebrates and plants have in the past received a totally inadequate proportion of exhibition space. It was therefore inevitable, and only right, that the new exhibits should be at the expense of the great range of extensive (though mainly old-fashioned) displays of living and fossil vertebrates. It is natural for vertebrate zoologists to regret this, but we should surely be reassured by Dr Miles' statement that the new exhibits "will contain the vast majority of the material now on show in the museum".

Yours faithfully,
BARRY COX

King's College, London

Links with school

SIR,—As a teacher of biology, I would like to offer my views about the recent article entitled 'Whither the Natural History Museum?' by Dr B. Halstead.

My earliest recollections of the museum are those of my childhood, when I was taken on the usual tour of the museums on a trip to London. I remember, most of all, the dinosaurs but little else except endless rooms of glass cabinets full of bones and a lot of unpronounceable words.

Today, thankfully, the presentation of material in the museum is progressing and the two most prominent examples of this are the new exhibitions of human biology and ecology. These offer a new and very important dimension to the role of the museum—namely a vital resource which links important aspects of natural science with what is being taught at school.

I have been lucky enough to use the exhibition of human biology as part of my exam course in the fourth and fifth years. The pupils concerned were taught how to use the exhibition and, as a result, were able to benefit from it in a way they could not by just relying on textbooks at school. I look forward to using the ecology exhibition in the same way with my second year groups next summer. The publications accompanying the two exhibitions also allow for follow-up work at school.

From Dr Miles' figures regarding visitors to the museum, it would appear that these exhibitions will benefit an age group whose presence is sadly missing from the museum.

I would like to say in conclusion that the museum should continue to present its rare and fascinating fossil exhibits, as it has done for years, thus fulfilling its role to the scholars; but also to continue with its new fresh approach and present more exhibitions which are of more use to schools and the less scholarly members of the general public.

Yours faithfully,
D. A. WEALE

St John's School,
Epping, Essex, UK

Teachers need a say

SIR,—I am a teacher in the fourth year of a large multi-cultural East London primary school. I am prompted to write in response to the articles by Dr Miles and Dr Halstead on the future of the British Museum (Natural History).

Dr Miles says that to some extent the Natural History Museum is for children under the age of eleven. He points to the absence of secondary school pupils and university students from the public galleries. Without doubt the primary school children are impressed by the large dinosaurs and blue whales. They take a passing interest in one or two other galleries or individual exhibits but generally scurry through the remainder of the museum if given the opportunity. To the children many of the current displaps seem unrelated to each other or to a wider theme I am not surprised that the majority of younger visitors are not motivated to return for many years.

On the other hand the recent human biology exhibition is stimulating and dynamic and makes demands of the children. In my experience they take an active interest in the displays and are keen to seek answers to questions. Furthermore the exhibition is coherent and so more meaningful to them. Although many of the concepts explained are beyond the grasp of the average eleven year old, I anticipate that a higher proportion are likely to revisit and develop their understanding than would have done had the exhibition remained in the traditional layout. If other recommendations in the paper 'A Proposal for a New Approach to the Visiting Public' are implemented I feel that many teachers and pupils will benefit even more.

It is disquieting to hear of the rift between the Department of Public Services and scientific staff Dr Halstead himself, however, refers to a false dichotomy, by discussing the former dinosaur gallery and its multi-level appeal. Surely the 'new style' exhibition can also be both stimulating and scholarly given the goodwill and collaboration of scientists and exhibition staff. After all Dr Miles does say that the exhibits to be displayed will contain the vast majority of the material on show.

Dr Halstead calls for the fullest possible public discussion. Hopefully teachers at all levels will be given an opportunity to participate in this discussion which at the moment seems to be conducted at the level of the academic.

Yours faithfully,
L. J. STEVENS

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Arborescent animals and other colonoids

SIR,—Comparative biology is sometimes hindered by terms which have become misleading with the advance of knowledge. 'Individual' and 'colony' are such terms when they are used to refer to a single polyp and the entire organism of a reef coral, for instance. The organism responds as a unit in growth and physiology, and it is also the unit of individual selection. I therefore propose two new general terms to replace 'individual' and 'colony' in such cases. Specific terms are already available in some instances, but they vary from taxon to taxon.

Individuoids are parts of an organism which have the general structure of whole free-living individuals but which connect with each other to form a coloniod. A colonoid usually has the same genes throughout and functions as a single individual.

The concept of colonoid grades into that of colony or association, which may be restricted to a group of proximate but physiologically separate individuals, usually of one clone. Intermediate cases might be grass swards or diatom chains. The concept of a colonoid organism also grades into that of individual *sensu stricto*, in which case individuoids may resemble organs. Examples are rooting branches of mangroves, bryozoan avicularise, or cyanophyte heterocysts. Slime mold colonoids are colonoids for only part of the life cycle and may form (sometimes necessarily so) from individuoids of different genotypes. Such fuzzy boundaries of concepts are necessary in the real world.

A diverse array of animals grow in a treelike form. Some but not all have apical meristems. Examples of arborescent growth occur among sponges, hydroids, alcyonarians, corals, bryozoans (including entoprocts[1]), graptolites, pterobranchs, and probably elsewhere. Comparative studies of their growth and that of branched plants and protozoans, from both mechanistic and adaptive viewpoints, have always proved illuminating in my seminar course on the evolution of development and would undoubtedly be more illuminating if pursued more fully.

The study of plants and arborescent animals can thus be mutually beneficial, but such study presupposes that functionally corresponding units are identified beforehand. Studies of other phenomena, such as adaptive strategies, also need clear recognition of what units are appropriate. This has not always been done, perhaps because of the pervasive shadow of a purely morphologically based terminology, and the result is then biological nonsense.

Yours faithfully,
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[1] Neilson, C. *Ophelia*, 9, 209-341; 1971.

news and views

Whaling: past, present and future

from Robert M. May

Few, if any, conservation movements have received as sustained and widespread support as those aimed at 'saving the whales'. Although most of us never have and never will see a great whale outside a museum, we are dismayed by the excessive exploitation these all-time largest of animals have suffered.

People who are vaguely aware of the sorry history whereby stock after stock of whales has been harvested well below the point of maximum sustainable yield (MSY) may be surprised to learn how things stand today. Of the twelve species of whale that have been hunted over the centuries, seven are completely protected in all oceans, three in most ocean areas, and the remaining two in some areas, under the current policies of the International Whaling Commission (IWC). There remain, of course, many problems, not the least being whaling activities under the flags of nations that are not IWC members, and the fact that even for whaling by IWC members the enforcement of agreed regulations leaves a lot to be desired.

The New Management Procedures of the IWC were adopted at its 26th meeting in 1974, and first put into effect in the southern summer of 1975–76 This procedure divides whale stocks in each of the ocean areas defined by the IWC into one of three categories, according to whether the populations are thought to be numerically bigger or smaller than, or near to, the size required to give MSY (for a more full account of the notion of MSY, see *News and Views* **263**, 91; 1976). This classification is revised annually, and more or less automatic rules then applied; if the stock is judged to be below the MSY level, a moratorium is declared ('protection stocks'); if it is near the MSY level, annual quotas are set with the intent of keeping the stock around this level ('sustained management stock'); if the estimated stock size is significantly above the MSY level, exploitation is permitted under quotas aimed to produce a controlled reduction toward the MSY level ('initial management stock'). Application of these rules in 1978 (and in the summer 1977–78 in the southern oceans) produced the numerical quotas shown in the maps. In addition to the wholly protected blue, grey, humpback, and four species of right whales, it will be seen that fin, sei and Bryde's whale are protected in most areas. Sperm whales have significant sexual dimorphism and a more elaborate social structure than the baleen whales, and for them separate quotas are set for males and females (see legend); the models used to obtain these numbers are currently being scrutinised and refined by the IWC.

Before considering the strengths and weaknesses of this management procedure, it is interesting to look at some of the historical background and at the tensions between economic interests and sustainable yield criteria.

History of whaling regulations

The whales that have been hunted include 11 species of baleen whales, which are filter-feeders with baleen plates ('whalebone') in place of teeth: (see box). The largest species of toothed whale, the sperm whale, *Physeter catodon*, is also hunted, and provides most of the present world catch by numbers and by weight (though not by value)

Formerly, these animals provided oil for lamps and lubricants, and the baleen whales also yielded whalebone. Baleen whales remain a source of oil for the manufacture of margarine, soap, and glycerine, of meat which is eaten directly by humans and pets, or used in soups and flavouring and of meat and bone meals for fertilisers and animal foods. Sperm whale oil and spermaceti provide industrial scouring and hardening agents, lubricants and cosmetics. As emphasised by many people, "all these products could be replaced by synthetic derivatives from vegetable and mineral sources, but it is still economically attractive to produce them from whales" (Gambell & Brown *IUCN Bull. Ser. II* **21**, 1; 1971).

Bowhead (Greenland right)	*Balaena mysticetus*
North Atlantic right	*Eubalaena glacials*
North Pacific right	*Eubalaena seiboldii*
Southern right	*Eubalaena australis*
Grey	*Eschrichtius robustus*
Blue	*Balaenoptera musculus*
Fin	*Balaenoptera physalus*
Sei	*Balaenoptera borealis*
Bryde's	*Balaenoptera edeni*
Minke	*Balaenoptera acutorostrata*
Humpback	*Megaptera novaeangliae*

The North Atlantic right whale was hunted by the Basques as early as the twelfth century. By the beginning of the twentieth century, the more easily catchable species—grey, three species of right, bowhead (the latter four species often lumped together as right whales)—were reduced to very low levels of abundance. These are all slow swimmers that usually float when dead, and they were pursued in open boats under oars or sail; real Moby Dick stuff. Whaling for the swifter swimming rorquals—blue, fin, sei, Bryde's and very recently minke—began in the mid-nineteenth century with the invention of the explosive harpoon, harpoon cannon, and the use of steam-powered ships It spread to the Antarctic in 1904, starting at South Georgia. The advent of the factory ship in the mid-1920s freed whalers from land stations, and began the modern era of Antarctic pelagic whaling. By 1935, fears that Antarctic stock would be depleted as Northern ones had earlier been culminated in the formation of an International Convention for the Regulation of Whaling, which banned all harvesting of right whales or of any female whales with calves.

World War II gave the whales a respite. In 1946, the pre-war threads were picked up by a new International Convention which established the IWC, charged to "provide for the conservation, development, and optimum utilisation of the whale resources, [taking]

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into consideration the interests of the consumers of whale products and the whaling industry". The grey whale was added to the protected list in 1947.

The Commission is advised by a Scientific Committee, which from early days has issued warnings and pleas that stocks were being exploited too heavily. The present analytical models and quantitative methods of population assessment derive mainly from the work of the Committee of Three Scientists (Allen, Chapman and Holt), appointed by the IWC in 1960. The reports of this committee in the early 1960s made it unanswerably clear that stocks of blue, fin, and humpback whales were being seriously overfished, and in 1963 they recommended total protection of blue and humpback, and a reduction of the Antarctic catch of fin to 5,000 whales or less. The IWC responded by partly protecting the blue and humpback, and reducing the Antarctic catch limit from 15,000 to 10,000 'Blue Whale Units' (this was an atrocious unit, whereby 1 BWU=1 blue, or 2 fin, or 2.5 humpback, or 6 sei) This pattern of the IWC reacting to the advice of its own Scientific Committee by doing too little, too late, persisted. Nonetheless, the 1960s and early 1970s saw some advances as the seeds of the New Management Procedures were sown. the Blue Whale Unit was abolished; two species were added to the list of those completely protected (the blue in the North Atlantic in 1960, the North Pacific in 1966 and world-wide in 1967, and the humpback in the North Atlantic in 1955, southern hemisphere in 1964, and world-wide in 1966): quotas for the Antarctic fin and sei were eventually set under the constraint that they be no more than the estimated sustainable yield.

Outside the IWC, pressures were building up. In 1966, the 1958 Geneva Convention on the Conservation of the Living Resources of the High Seas came into force. adjuring "measures rendering possible the optimum sustainable yield . . . so as to secure a maximum supply of food and other marine products". The UN Conference on the Human Environment, held in Stockholm in 1972, recommended a 10 year moratorium on all commercial whaling. and this recommendation was subsequently adopted by the UN General Assembly and by the General Assembly of the International Union for the Conservation of Nature (IUCN).

The IWC considered this recommendation at its meeting in 1974, but settled for adopting the New Management Procedures described above. The technical details were hammered out in 1975, and led to the following definitions. A protection stock is one which is more than 10% of MSY stock level (MSYL) below the MSY level itself. A sustained management stock is one that is not more than 10% of MSYL below, nor 20% of MSYL above, the MSY level. Finally, an initial management stock is one which is estimated to lie more than 20% of MSYL above MSYL. Thus, for example, if the stock obeyed a logistic recruitment curve, and if the virgin stock density were defined to be 1, then a stock density of less than 0.45 would be protected, between 0.45 and 0.60 would be sustained management, and greater than 0.60 would be initial management The rules also embody some caution with respect to possible errors in the estimates of MSY levels; no more than 90% of the estimated MSY can be taken, regardless of the stock class. Furthermore, for initial management stock, not more than 5% of the estimated initial exploitable stock is to be taken in any one year, unless there is 'positive evidence' that higher levels will not lead to over-exploitation. Nor may significant exploitation begin until a satisfactory estimate of stock size has been obtained. (For more full accounts, see Brown *Polar Record* **18**, 85; 1976; **19**, 59; 1978; *FAO Fisheries Report* No. 194 (the Report of the Advisory Committee on Marine Resources Research, chaired by Laws); 1977.)

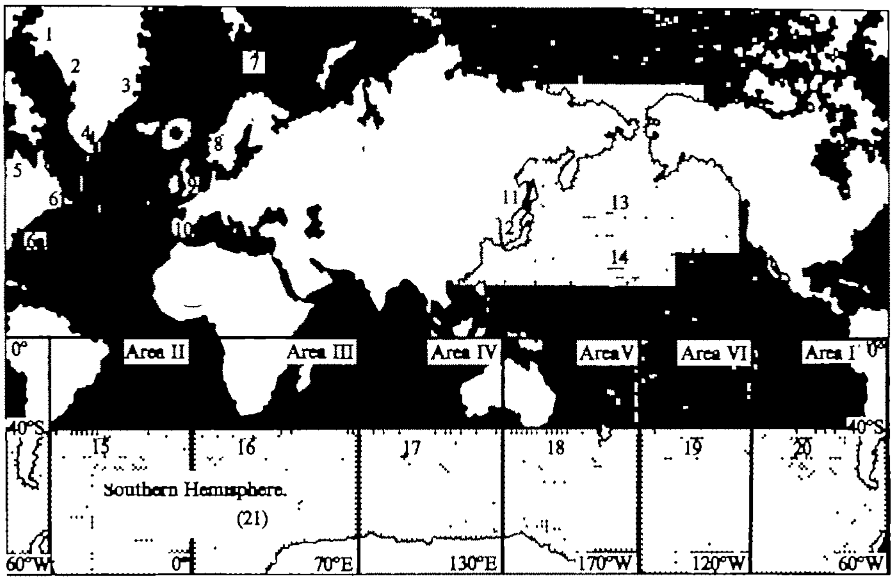

Fig. 1 Quotas for baleen whale species in the various ocean areas designated by the IWC (1977/8 and 1978 seasons). PS stands for protection stock, SMS for sustained management stock, and IMS for initial management stock, as discussed more fully in the text. (1) West Greenland: minke (SMS) 397*, fin (SMS) 4*. (2) Iceland-Denmark Strait: sei (SMS) 84. (3) East Greenland-Iceland: fin (SMS) 304 Not more than 1,524, 1977-1982. (4) East Greenland-Iceland-Jan Mayen: minke (SMS) 320. (5) Canadian East Coast: minke (SMS) 48. (6) Newfoundland-Labrador: fin (IMS) 90. (6a) Nova Scotia: fin (PS) 0; sei (PS) 0. (7) North Norway: fin (SMS) 61*. (8) West Norway-Faroe Islands: fin (PS) 0. (9) Svalbard-Norway-British Isles: minke (SMS) 1,790. (10) Spain-Portugal-British Isles: fin (SMS)*. (11) Okhotsk Sea, West Pacific: minke (SMS) 400. (12) Sea of Japan: minke (SMS) –. (13) North Pacific: fin, sei (PS) 0, Bryde's (IMS) 524. (14) Remainder: minke (IMS) 0 pending a satisfactory estimate of stock size. (15) Sei (PS) 0, minke, (–) 1,150. (16) Sei (PS) 0, minke (–) 1,826. (17) Sei (SMS) 460, minke (–) 963. (18) Sei (PS) 0, minke (–) 930. (19) Sei (PS) 0, minke (–) 688. (20) Sei (SMS) 388, minke (–) 704. (21) fin (PS) 0; Bryde's (IMS) 0 pending a satisfactory estimate of stock size; sei, catch not to exceed a total of 771; minke, catch not to exceed a total of 5,690.

MSY *versus* economic considerations

Reviewing the above history, Gambell and Brown (*op. cit.*) note that "despite warnings from the scientists that the catches were considerably larger than the stocks could withstand in the long term, the whaling nations allowed their industries to take almost as many whales as they could, arguing that they could not otherwise survive economically They leaned on the fact that the scientific evidence was not complete, precise and incontrovertible". The basic reason for this behaviour is that, although the discussions formally centre around sustainable yield (with present and future catches in principle regarded equally), the industry is more interested in economic returns. This tension between MSY principles and economic actualities takes many forms, some of which are widely appreciated and others not; the general issues have been set out incisively by Clark (*Mathematical Bioeconomics*, Wiley, 1976).

The first problem lies in establishing some agency that is effectively the sole owner of the resource. Failing this, an open-access fishery suffers a 'tragedy of the commons' (Hardin *Science* **162**,

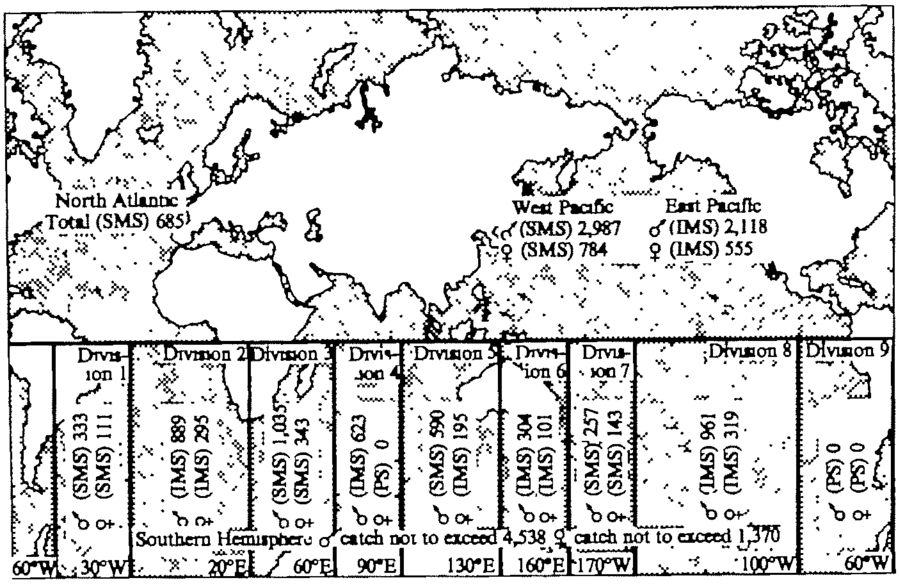

Fig. 2 IWC quotas for sperm whales (1977/78 and 1978 seasons); there are separate quotas for males and females.

1243; 1968), in which the 'economic rent' is dissipated by exploitation down to the point where revenues are balanced by harvesting costs. The establishment of the IWC, which aims to act as such a sole owner, is thus a non-trivial accomplishment for which its members deserve credit. It is in the nature of the situation that there remains a tendency for individual ships (and even—whisper it—nations) to cheat.

Moreover, as stressed by Clark (see also *News and Views op. cit.*), whalers are interested not in maximising sustainable biological yields, but in maximising the present value (PV) of net economic return, discounting the future. If the economic discount rate is small compared with the biological growth rate of the exploited stock, these considerations are relatively unimportant, and MSY and PV criteria lie close. But if the economic discount rate is greater than, or of the order of, the population's intrinsic growth rate (as arguably is the case for whales, where growth rates lie around 5 to 10% per annum), the PV criterion will tend to lead to stocks being depleted below the MSY level. Indeed in the limit when the discount rate becomes infinite (which in practice means when it significantly exceeds the biological growth rate) exploitation by a sole owner is mathematically identical with the open-access case. The reason is intuitively clear; with relatively high discount rates there is, just as in open-access exploitation, no motive to heed the future These remarks are subject to many qualifications, among them that prices, harvesting costs, and inflation-corrected discount rates are all liable to change over time (Clark, *op. cit.;* Gulland, *Nature* **263**, 722; 1976; Clark & Munro *J. Env. Econ. Mgmt.* **5**, 198; 1978). All this can make for great complications in the analysis, but I think the essentials are as above.

These economic considerations have some subtle corollaries which are not so widely acknowledged. Clark and Munro (*J. Env. Econ. Mgmt.* **2**, 92; 1975), for example, have shown that the optimum trajectory for capital investment can, under some circumstances, lead to an initial rapid phase of heavy exploitation, possibly even depressing stock below the level that maximises PV (much less SY), followed by a slower phase in which the stock levels recover to and are maintained around the PV level. Very crudely speaking, these two phases correspond to fast capitalisation followed by relatively slow amortisation of the equipment.

Even within an agreed MSY framework, there is room for squabbles motivated by economics. One such dispute at present concerns whether the quotas for sperm whales should be set for MSY by numbers or by weight. It happens that for sperm whales a significant fraction of the life-long growth in size takes place during the exploited adult phase of the life cycle. The result is that, once the equilibrium age structure is attained under a given harvesting regime, MSY by weight gives a total catch greater in biomass (and thus in products) and lower in numbers (therefore requiring less effort and lower harvesting costs) than is obtained by MSY based on numbers (Holt, FAO Document ACMRR/MM/SC/99; 1976). Unambiguously, the long term advantage lies with MSY by weight. The snag is that several years must elapse before sperm whale populations change from the existing age structure, produced by harvesting for MSY by numbers, to that appropriate to the MSY weight criterion. If the future is heavily discounted, one will always argue for the numbers criterion, because this will allow more whales to be caught, and, in the immediate future, their average weights will be much the same under either quota system. In this way can the future be squandered even in an MSY context.

One bleak consolation lurking in the above discussion is the possibility that whaling, once it is effectively controlled at MSY (as opposed to maximum PV) levels, may cease to be economically attractive. This issue, however, requires taking a longer view of prices and costs than I am prepared to do.

Prospects for the whales

The present biomass of large whales is estimated to be about 23 million tons, which is about one-third the value around 1900 (FAO *op. cit.*). Some species have, of course, suffered very heavily; for example, the populations of blue and humpback are down to a few percent of their pristine numbers. However, no species has been extinguished by whaling, although the grey whale is extinct in the North Atlantic.

Ironically, the one species that could conceivably be in danger of extinction is the bowhead. Although this species has long had protection status, up to 1978 the IWC rules permitted hunting by aboriginal peoples. Recent increases in the prosperity of societies of Alaskan Eskimos have resulted in their hunting with more boats, which seem on the average to be less ably manned. Not only have the number of bowhead caught increased markedly over the past decade (from an average around 9 per annum in 1912-1967 to around 27 over the past decade: Chapman, *Marine Mammal Information*, Dec 1977), but the number struck and lost has greatly increased. It is likely, although in dispute, that the number of animals thus killed exceeds the number actually harvested. Conservative estimates suggest the present levels of exploitation exceed the rate of net reproduction. The sentimental argument that these people should be allowed to continue their depredations, lest their traditional culture be imperilled, seems to me to miss the point that the current problems arise largely because the traditional culture is already collapsing.

The only stock that has demonstrably recovered under protection is the grey whale in the eastern North Pacific (the California grey whale). Some humpback and some right whale populations show indications of increases in recent years. It is still too early to detect with assurance whether blue whale stocks have been increasing since they were protected. The uncertainties here derive from the difficulties in getting enough survey data to measure significant changes in abundance.

The New Management Procedures have the virtues of recognising individual species and geographical stock units, each subject to its own management regime, and of classifying stock with the explicit aim of long term conservation. The allowance for a margin of error in the estimation of MSY level is another step in the right direction; this precaution helps offset uncertainties in the determination of population parameters, and gives some buffering against the unpredictable environmental fluctuations to which all natural populations are subject to one degree or another. The procedures thus go some way toward meeting the broad principles for conservation and management articulated in the so-called Airlie proposals, which emerged from a consultation sponsored by the World Wildlife Fund, the Ecological Society of America, the IUCN and others (see Holt & Talbot *Wildlife Monogr.* No. 59; 1978).

The New Management Procedures could be improved in at least three ways. First, they could explicitly acknowledge the long time lags inherent in the dynamics of whale populations, and the problems these cause. Second, they should aim to maximise the net sustainable yield of whale products rather than the number of whales caught; that is, they should take full account of whaling costs, and should maximise the catch by weight not by numbers. Finally, and most difficult, it needs to be recognised that whale stocks are not single species, but are constituents of a multispecies ecosystem. A management procedure that (as required by the US Marine Mammal Protection Act of 1972) takes due account of the 'health and stability of the ecosystem' will need to grapple both with technical issues of multispecies MSY criteria, and with broad ecological questions of the possible existence of alternative stable states.

Immunoglobin genes: more facts to guide speculation

from W. A. Dunnick

BIOLOGISTS have proposed all sorts of genetic gymnastics for immunoglobulin genes. They have suggested that immunoglobulin genes hop around chromosomes, and recombine or mutate at terrifying rates. The genes have been amplified, branched, interrupted by episomes, and somatically diversified, but never understood. Immunoglobulin genes attract this bizarre speculation because of the protein's enigmatic structure.

Both the light and heavy chains found in immunoglobulin proteins have structurally simple carboxtermini. For example, the carboxy halves (C-regions) of about 95% of mouse light chains have an identical 'kappa' sequence. The remaining 5% are divided between two related 'lambda' sequences. On the other hand, the amino terminus (V-region) is very heterogeneous in sequence. Individual mice may be able to express 10,000 different V-regions. The variability is not random, but is concentrated in three 'hypervariable' regions. No other group of polypeptide sequences implies such an extensive, yet nonrandom, divergence of genes. Most speculation concerning immunoglobulin genes centres on two problems: (i) the bringing together of the very different V and C sequences to form a single polypeptide, and (ii) the generation of V-region diversity.

V–C joining

If each of the 10,000 V-genes were associated with a C-gene, the first problem would be solved. However genetic and gene counting experiments have shown not thousands, but only a few C-genes. Thus, this simple model must be excluded.

A second simple model would be the association of a few C-genes with a few V-genes. Mutations in the V-genes during somatic development could give rise to 10,000 different V sequences (in 10,000 different cells) associated with a single C sequence. However, there seem to be more than a few germline V-genes. V-regions are grouped according to shared amino acid sequences; members of a single subgroup share three or more residues not found in other subgroups. To avoid the unpalatable assumption of parallel mutation, each subgroup is thought to be coded by at least one germline gene. Such an analysis implies that the few kappa C-genes must accommodate 50 or more kappa V-genes.

Dreyer and Bennet (*Proc. natn. Acad. Sci. U.S.A.* **54**, 864; 1965) proposed that V and C-genes are brought together by a translocation event, cells expressing different V-regions rearranging different V-genes. Hozumi and Tonegawa (*Proc. natn. Acad. Sci. U.S.A.* **73**, 3628; 1976) demonstrated such a rearrangement for a mouse kappa chain (see *News and Views* **264**, 699; 1976), and rearrangement of V and C-genes has also been shown for a second kappa chain (Rabbitts & Forster *Cell* **13**, 319; 1978) and for a lambda chain (Tonegawa *et al. Cold Spring Harbor Symp. quant. Biol.* **41** 877; 1976).

These observations suggested that translocation results in the contiguous V–C sequence found in mRNA (Milstein *et al. Nature* **252**, 354; 1974). It was soon demonstrated that this is not the case. Nucleic acid hybrids between kappa cDNA (Rabbitts & Forster, *op. cit.*) or mRNA (Matthyssens & Tonegawa *Nature* **273**, 763; 1978) and their respective myeloma genes have looped-out, single-stranded regions that are susceptible to S1 nuclease. The size of the products of S1 digestion suggests that the discontinuity in the gene is near the V–C junction. Also, electron microscopy of R-loops formed between lambda mRNA and a cloned myeloma V+C gene reveal a 1,200 base pair intervening sequence between the V and C coding sequences (Brack & Tonegawa *Proc. natn. Acad. Sci. U.S.A.* **74**, 5652; 1977).

The complete structure of the mouse lambda germline gene (Bernard, Hozumi & Tonegawa *Cell*, in the press) has now been determined. It consists of 45 bp coding for the lambda signal peptide, 306 bp of variable region, more than 4,500 bp of undefined DNA, 39 bp of variable region sequence, approximately 1,200 bp of intervening sequence, 312 bp of constant region sequence. (The preceding implies that the V-gene is 'upstream' relative to the C-gene; this is not known to be the case.) In cells producing lambda light chains, a translocation event fuses the two V segments, eliminating the large undefined segment of DNA from the active gene. The small segment of 'variable' region associated with the C-gene (albeit 1,200 bp away) is a surprise. Bernard *et al.* have termed the segment J, emphasising its role in V–C joining. J. Seidman and colleagues (NIH) reported a similar structure for a kappa gene at the Seventh Workshop on Homogenous Antibodies*.

We are left with the V and C-genes over 1,000 bp apart in myeloma DNA,

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*Held at the National Institutes of Health, 23–25 October.

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but contiguous in mRNA. Three lines of evidence indicate that nuclear RNA precursors (Gilmore-Hebert & Wall *Proc. natn. Acad. Sci. U.S.A.* **75**, 342; 1978; see also Schibler, Marcu & Perry *Cell* in the press) contain these intervening sequences. First, hybrids between cDNA and the precursor RNA are not perfect matches, but have single-stranded regions near the V–C junction (Rabbitts *Nature* **275**, 291; 1978). Second, the precursor and mRNA apparently have the same nucleotide sequence in the C-region. On the 5′ side of the C-region, sequences in the precursor differ from those in the mRNA (Rabbitts *op. cit.*). Third, ultraviolet inactivation experiments suggest that V and C coding sequences are not adjacent in nuclear RNA (R. Wall & M. Gilmore-Hebert, University of California, Los Angeles, reported at Homogenous Antibodies Workshop). The intervening sequences in nuclear RNA must be removed by some RNA processing mechanism.

Single cells produce only one sort of light chain, but seem to be able to couple one heavy chain V-region with two or more heavy chain C-regions (for example, mu and gamma C-regions). Several authors have suggested that the heavy chain V-region may be cotranscribed with several C-regions, and that RNA processing would determine which heavy chain V–C combination(s) is to be expressed. This elegantly simple idea will have to be revised; hybrid cells producing two different classes of heavy chain do not exchange V and C-regions (Milstein *et al. Cold Spring Harbor Symp. quant. Biol.* **41**, 793; 1977; Köhler & Schulman *Nature* **274**, 917; 1978). For example, hypothetical 'mu' processing enzymes do not bring together the mu constant region and a variable region associated with a gamma constant region, even though both genes are transcribed in the same hybrid cell. In addition, R. Perry, U. Schibler and K. Marcu (Institute for Cancer Research, Philadelphia) reported at the Homogenous Antibodies Workshop that both gamma and alpha heavy chain mRNAs are transcribed as precursors at least 8 to 11 kb long. Nevertheless, neither mu nor alpha transcripts are detected in the nucleus of the gamma producer, and neither mu nor gamma transcripts are found in the nucleus of the alpha producer. Thus, the mu-gamma-alpha switch cannot be explained by a simple RNA processing model (for an alternative theory, see Honjo & Kataoka, *Proc. natn. Acad. Sci. U.S.A.* **75**, 2140; 1978).

V-region diversity

The extraordinary results discussed above have solved, in a general way, many of the problems of light chain V–C joining. In relation to V-region diversity, many immunologists believe that some number of germline V-genes (say 100 to 1,000) diversify somatically to yield a larger number of total genes (10,000). However, this is not to say that all immunologists endorse such a statement; the gap between belief and proof has been great. Kinetics of hybridisation experiments have suggested a limited number of germline V-genes, but the interpretation of such data is very difficult (see Smith *Cold Spring Harbor Symp. quant. Biol.* **41**, 863; 1977; Rabbitts & Milstein *Cont. Topics Molec. Immun.* **6**, 117; 1977).

The extensive study of one kappa group (V21) has been more informative than investigations of the widely diverse kappa chains as a whole, or studies of kappa groups in which only a few amino acid sequences are known. The V21 group may be divided into at least six subgroups on the basis of related sequence patterns (McKean *et al. Proc. natn. Acad Sci. U.S.A.* **75**, 3913; 1978; Weigert *et al. Nature*, in the press). The subgroup structure is most clear if residues 99–110 (J-region) of the V-regions are ignored. The V21 subgroups seem to randomly associate with eight J-regions (Weigert *et al. op. cit.*) As Bernard *et al.* (*op. cit.*) proposed, V-region diversity may increase through recombination of V and J-regions or through imperfect V-J joining. Using a hybridisation technique (saturation) which should be more accurate than kinetic measurements, Valbuenna *et al.* (*Nature*, in press) have suggested that the number of V21 germline genes is the same as the number of subgroups (four to six). This would strongly favour somatic variation in this group, since nearly 30 amino acid sequences have been determined. Impurities in the cDNA preparation complicate the interpretation of these results, and one eagerly awaits the results of such experiments with cloned cDNAs

Seidman *et al.* (*Science* **202**, 11; 1978) have also suggested that mouse kappa V-genes are represented in the germline by groups of at least six closely related genes. Two-dimensional 'fingerprints' of mouse DNA reveal six to ten bands of hybridisation to each of two different cloned kappa probes. Even though the two probes identify the same C-region band, they have no V bands in common. The authors speculate that the total number of kappa germline V-genes is 200 to 1,000. Experiments with many light chain probes will be necessary to reach a more precise estimate.

Cloning and sequencing of two V-genes hybridising to the MOPC 149 kappa probe have demonstrated that the two genes vary from each other and from the MOPC 149 V-gene by about 7%. The two V-genes are also homologous over 2,500 bp of flanking sequence. Seidman *et al.* expand an earlier hypothesis of Edelman and Gally (*Proc. natn. Acad. Sci. U.S.A.* **57**, 353; 1967). V-region diversity is expanded by recombination, the recombination being facilitated by the homology between flanking regions of different V-genes. The hypothesis is especially persuasive, since other structural genes (β-globins) diverge more slowly, and have less flanking region homology.

In spite of the abundance of remarkable data, our concepts of immunoglobulin genes remain vague in several areas. Are any special mutational mechanisms required to generate V diversity? How are heavy chain genes rearranged and joined? What is the molecular basis of immunological memory and tolerance? And finally, cellular immunologists spend more time with T cells than with immunoglobulin-producing cells these days. How do T-cell receptor genes relate to Ig genes. ☐

Cosmic spherules

from D. W. Parkin

MOST scientists will not have heard of cosmic spherules, yet Murray and Renard found and named them long ago (*"Challenger" Reports* **4**, 1891). About a dozen of these extraterrestrial spherules, ~ 100 μm diameter, can be magnetically extracted from a cupful of deep oceanic red-clay, which slowly deposits at a few metres per 10^6 years They are black and quite spherical. Two types occur: the 'stones' and the 'irons', although a few might be called 'stony-irons'. The stones are olivine $(Mg, Fe)_2SiO_4$ and magnetic Fe_3O_4; a little glass may be present but not as a noticeable phase. The irons are magnetite and wüstite FeO; often a globule of Fe/Ni alloy is present, eccentrically placed in the oxide shell. There are other rounded magnetite forms in the red-clay which are not extraterrestrial. These occur especially in the less than 30 μm-sized range Probably these faceted and very shiny black forms are growths on the sea bed or may come from volcanoes. Care is needed not to confuse these small particles with cosmic spherules. Above 50 μm there is little chance of contaminating a collection. The fact that the ratio of stones to irons is the same in any surface sample of red-clay provides a strong argument for an extraterrestrial source for both types of spherules.

Most meteorite experts would accept the extraterrestrial origin; but they

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have tended to ignore cosmic spherules, believing that they are merely droplets sprayed from a meteorite as it ablates in the atmosphere. On the other hand, Opik (*Irish Astr. Jour.* **1**, 145; 1951; **4**, 84; 1956) proposed a more interesting origin: cosmic spherules are formed in the atmosphere by the melting of similarly sized fragments coming directly from the zodiacal cloud. Using his micrometeorite formula, he was able to predict size-distributions for cosmic spherules. Unfortunately, when the predicted and observed distributions are compared, the fit is not exact. Most zodiacal cloud particles are known to orbit the Sun with low inclination to the plane of the ecliptic, but it is not known how many of them depart from circular orbits. Therefore, there is some flexibility in the allowed velocity of impact with the Earth's upper atmosphere; also the shape of the impacting fragment enters into the theory. With contrived and somewhat artificial mixtures of velocities and/or shapes, a better fit can be achieved. Hunter and Parkin (*Proc. Roy. Soc.* **A255**, 382; 1960) largely avoided these complications by comparing the size distributions of stones with irons and they considered that Opik's zodiacal hypothesis was essentially correct, but the argument was not convincing. Meanwhile, to prove a zodiacal origin, interest was centred on making day by day airborne collections of cosmic spherules at remote sites; but these promising activities soon died away. The collections were always contaminated by industrial magnetic spherules. During the 1960s no positive progress favouring the zodiacal origin could be made; the rather uninteresting meteoritic ablation mode of formation was the only idea that prevailed.

Now, as a result of a few recent papers, interest in cosmic spherules is likely to be renewed. Brownlee *et al.* (*Interplanetary Dust and Zodiacal Light*, Eds. H. Elsässer and H. Fechtig, Springer-Verlag, 279, 1976) have painstakingly identified extraterrestrial dust by using aircraft in the stratosphere. Amongst these μm-sized crumbs, most of which are like carbonaceous meteoritic material, similar sized spherules have been found at about 10% frequency. It could be that both crumbs and spherules are coming directly from the zodiacal cloud. Parkin *et al.* (*Nature* **266**, 515; 1977), using scanning electron microscopy and X-ray diffraction on cosmic spherules, were persuaded that spherules are not meteoritic ablation droplets. From their surface texture, which shows only slight but varying atmospheric damage, it was claimed that cosmic spherules are already round bodies in space. With these ideas of non-molten (grazing) atmospheric flight, Opik's theory can be used to give a much more convincing explanation of the size-distributions, stones and irons being treated separately. If a spherule (on a plunging flight) was heated to melting temperatures, the size distribution analysis predicts that it would be destroyed; probably due to evolution of solar-wind gases accumulated during the spherule's residence in space. Very recently, Ganapathy *et al.* (*Science* **201**, 119; 1978) have analysed three stony spherules for trace elements, using neutron activation techniques. Not only do these analyses remove any lingering doubts about the extraterrestrial nature of stony spherules, but they break new ground in the chemical classification of cosmic spherules. This will become especially evident when more are analysed.

Of the eight elements analysed (Ir, Ru, Sc, Fe, Co, Ni, Cr, and Au) the amounts of Ir, Ru, Ni and Cr differ widely across the three spherules. Extra elements Zn, Sr, Os, Sb, Cs, Ag and Se are given for the third spherule. The authors also mention that S is highly depleted in these and other spherules. When the third spherule is compared with carbonaceous meteorite (C1) agreement is good, except for Au, Cs, Zn, and Ag which show moderate depletion. High depletion of very volatile Se and S is readily understood: the spherules have been molten at some time in their history. It is tempting (as the authors do) to compare cosmic spherules with C1 meteorites; and to attempt to derive each spherule from some primitive and homogeneous material, explaining the depletions and differences on the grounds of volatilisation on melting and leaching on residence in the sea water. But there is no compelling reason to do this for every spherule. It might turn out, when more spherules are analysed, that distinct families appear; with each family relating to the different meteorite types. It could even be that cosmic spherules are sparks from colliding meteorites or asteroids, the detailed chemical differences amongst them reflecting differences in the colliding bodies. Volatilisation of the moderately volatile elements or leaching of the soluble elements may not be as serious as Ganapathy *et al.* suspect—Cs is not highly depleted. In fact, we believe that most stony spherules in the surface sediments have not been severely attacked by sea water; the pores between the crystal grains being a natural freezing feature.

However, whatever the outcome of all this recent work, cosmic spherules are likely to become more widely talked about in future years. A pressing need is to find out where in the Solar System they are being created. This may be possible by examining deep-sea cores and using the fact that radiation from the Sun causes a drag on the orbiting spherule, the so-called Poynting–Robertson effect. Initial results from a red-clay core from the Pacific suggest the asteroidal belt. □

Muon spin rotation

from G. C. Stirling

ONE test of the public recognition of a new scientific technique is its emergence in the chemical sciences: optical spectroscopy, magnetic resonance spectroscopy and Mössbauer spectroscopy are common examples which have crossed the frontier from research curiosities in physics to routine tools in chemistry. The latest candidate is notable in representing spin-off from particle physics, a frequently-invoked, if trivial, justification for the vast funding accorded high energy physics research in recent years.

μSR—the acronym stands for Muon Spin Rotation, Relaxation, Resonance, Research or what have you, and refers to the analogy with NMR and ESR—has now come of age in this way after a relatively short adolescence, and at the recently-held first international meeting* on the subject much of the most promising new work related to purely chemical problems Indeed, the use of muons in chemistry is striking not only for providing yet another probe of chemical systems, but for opening up an entirely new field of study—muonium chemistry—with important implications for related disciplines.

The μSR method in itself is conceptually simple, and derives from various serendipitous properties of the muon: it has a mass about 200 times that of the electron, a lifetime of 2.2 μs, and muon beams are inherently spin-polarised. Muons are rapidly stopped in bulk matter, and the spin direction is maintained during the thermalisation process. Subsequent interaction with local internal fields or with externally applied fields causes the muon spin to undergo a Larmor precession, and it is the precise behaviour of this precession which provides detailed information on the local muon environment. The precession is readily observed by the spontaneous muon

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*First International Topical Meeting on Muon Spin Rotation, held at Rorschach, Switzerland, 4–7 September 1978.

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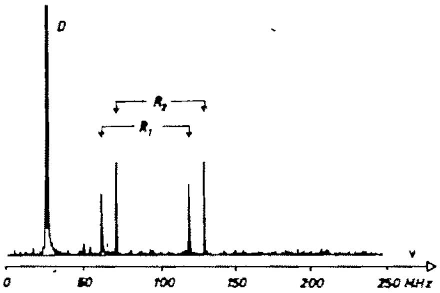

Fig. 1 Muon procession frequencies derived from the measured μSR time-histogram for 2-methyl-1,3-butadiene, with an external field of 1 kG. R_1 and R_2 originate from muonium-substituted radicals (after Roduner *et al., Chem. Phys. Lett.* **57**, 37; 1978).

decay, which leads preferentially to positron emission along the instantaneous muon spin direction, and appropriately located positron detectors give a direct observation of the time evolution of the muon polarisation

Chemically the most interesting possibility is the formation and subsequent behaviour of the atomic species muonium ($\mathrm{Mu} \equiv \mu^+ e^-$) Muonium has a similar size and ionisation potential to the hydrogen atom, and a mass some nine times less It therefore would be expected to behave like a superlight hydrogen isotope, providing possibilities for observing dramatic H/Mu isotope effects in chemical reaction kinetic studies (compared with the relatively staid hydrogen-deuterium difference) Indeed, measurements of rate constants for the reaction $\mathrm{Mu}+\mathrm{Br}_2$ is the gas phase (Fleming *et al., J. Chem. Phys.* **64**, 1281; 1976), and Mu in aqueous solutions (Percival *et al., Chem. Phys. Lett.* **47**, 11; 1977; Jean *et al*, *Chem. Phys Lett.* **57**, 293; 1978) have demonstrated the chemical similarity of H and Mu, although a detailed interpretation of observed isotope effects has been frustrated by the realisation that much of the previously accepted H atom data must now be reassessed. A new development reported at the Rorschach meeting concerns the direct observation of muonium-substituted radicals in solution Although measurement of muonium rate constants obviously may imply the formation of such radicals, it is only recently that they have been observed directly in a μSR experiment (Roduner *et al., Chem. Phys. Lett.* **57**, 37; 1978) In muonium-substituted radicals the observed frequency spectrum is dependent on the coupling of the muon spin with those of nearby nuclei and unpaired electrons, leading to characteristic spectra. A typical example is shown in Fig. 1, corresponding to radicals $\mathrm{CH_2{=}C(CH_3)CHCH_2Mu}$ and $\mathrm{CH_2MuC(CH_3)CH{=}CH_2}$. Hyperfine coupling parameters are derived from the spectra, and the large isotope effects compared with the analogous hydrogenous radicals are related to equilibrium structure and vibrational and rotational motions of the radical species. The implications of this work for reaction rate theory and radiation chemistry are substantial, the importance of the μSR technique lying in its unique sensitivity—since only one muon may be in the sample at any one time, reactions are pseudo-first order, and the reacting muonium species can in principle be identified unambiguously from its depolarisation behaviour

Why then, this apparently sudden activity in condensed matter applications of the muon? The answer undoubtedly lies in the availability of high quality muon beams at the new meson factories in North America, the USSR and Western Europe (though not, one notes, in the UK, although the proton synchrotron of the so-called spallation neutron source at the Rutherford Laboratory may yet provide a world ranking source). A striking feature of these facilities is the overall tendency towards 'applied' research, most markedly demonstrated by the Muon Spin Rotation technique itself. At the recent meeting for example, investigations using μSR included, in addition to purely chemical work, studies of the electronic structure of impurities in metals and magnetic materials, the behaviour of μ^+ and muonium in insulators and semiconductors, and the study of defects in radiation-damaged materials. As usual the biochemists have been quick to assess the potential of the technique in their own work. The proceedings are to be published in a volume of *Hyperfine Interactions* early in the new year, and this will doubtless serve to acquaint a larger audience with the possibilities of the technique for the first time Already preliminary plans are in hand for a second international meeting, most probably to be held in Vancouver in 1980 ☐

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Superfluid ^{3}He in narrow cylinders

from Dieter Vollhardt

SUPERFLUID ^{3}He is a fascinating liquid—it has an unusually large number of internal degrees of freedom, it is inherently anisotropic and displays uncommon magnetic properties There exist three superfluid phases. A, A_1 and a B phase which all differ in their spin-configurations. The A phase, in particular, can be characterised by two unit vectors: (1) the vector **l**, which describes the angular momentum of a ^{3}He–^{3}He Cooper pair and which is the anisotropy axis affecting, for example, flow motion of the liquid, and (2) the vector **d**, describing the spin part of the underlying wavefunction (if S is the total spin of a Cooper pair then $\mathbf{d}{\cdot}S$ is a constant of motion).

Both vectors are thus internally defined directions, which characterise the degrees of freedom of the superfluid and which will form vector fields defined throughout the whole sample Given a container and external fields, **l** and **d** will form a configuration such as to minimise the energy of the system. For this to occur several conditions, which usually all compete with each other, have to be optimised: (1) **l** must be perpendicular to any wall, (2) **d** and **l** want to be as straight as possible because bending costs energy; (3) **d** wants to be perpendicular to an external magnetic field because of the susceptibility anisotropy and finally **l** and **d** want to be parallel due to the nuclear dipole interaction in a ^{3}He–^{3}He pair. It is this very energy that leads to the unusual magnetic properties of superfluid ^{3}He, which become particularly evident in nuclear magnetic resonance (NMR) experiments. The bulk A phase hence shows not only a transverse resonance frequency which is strongly shifted away from the Larmor frequency but also a longitudinal resonance! By means of NMR experiments one can then indirectly obtain information about the **l** and **d** configuration in a sample, because **d** will oscillate in the potential provided by the **l**-vector, giving rise to particular resonances Of all textures the coupled **l** and **d** fields can build up, those which are non-uniform and show textural defects and singularities are of particular interest Quite generally the topics of defects and singularities in condensed matter physics and their classification has been of great theoretical interest for the past 2 years (see, for example, Toulouze & Kleman, *J Phys. Lett.* (*Paris*) **37**, L-149; 1976).

While some experimental work on non-uniform textures (without singularities) has been done already the first experimental studies which specifically deal with singular textures have only recently been reported (Saunders *et al. Phys. Rev. Lett.* **40**, 1278; 1978; Gould & Lee, *Phys. Rev Lett.* **41**, 967; 1978).

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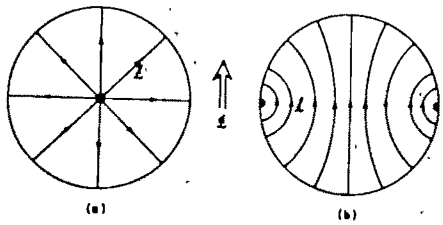

Fig. 1 Two possible planar textures of the **l** vector confined in a long cylinder *a*, Modified de Gennes disgyration with the **l** vector strictly radial, giving a singularity in the centre, and the **d** vector uniform. *b*, The 'Pan-Am' texture with two singularities on the walls. **d** is again uniform (from Gould and Lee, *op. cit.*).

By a rare coincidence both groups carried out NMR measurements of A-phase textures in the same type of very narrow cylinders (2 μm diameter). In the experiments an external static magnetic field was applied parallel to the cylinder axes with rf fields parallel and transverse to this to measure the longitudinal and transverse resonances. In addition, Gould and Lee also reported measurements of a transverse resonance with the external magnetic field perpendicular to the cylinders. The confinement of the superfluid in these narrow cylinders has drastic consequences for possible **d** and **l** textures. As bending of **l** and **d** usually takes place over a distance of typically 10 μm, such a deformation will cost quite a lot of energy if it happens over a length of 2 μm, even more energy than to have a singularity somewhere in the plane of the cylinder. The only known non-singular texture that would fit into a cylinder, the so-called Mermin-Ho vortex, can be shown to require too much bending energy to be a favourable candidate, so that the only possible textures must bear singularities. With a magnetic field parallel to the cylinder one would thus *a priori* expect planar textures with **d** uniform across the sample (in the plane of the cylinder cross section) and the **l**-field looking somewhat as in Fig. 1.

Measuring the transverse NMR signal, both groups find two resonances where initially most of the weight lies in the higher frequency mode; however, this mode slowly decays into the lower mode over 40–60 min, which seems to imply a textural transition. The two groups measured somewhat different NMR frequencies and while the resonances of Gould and Lee proved to be temperature dependent (suggesting a temperature-dependent texture) the one of Saunders *et al.* did not depend on temperature Their results for the longitudinal resonances are also different. Gould and Lee find a single, well defined, longitudinal resonance (temperature dependent) which does not change during the textural transition, while Saunders *et al.* do not observe any such resonance, which would be consistent with an axially symmetric texture. Whether the differences in the results can be explained by the different experimental setups (Gould and Lee for example, use Pomeranchuk cooling, Saunders *et al.* use nuclear demagnetisation) is unclear. The results, however, lead to the following conclusion. in both experiments the first texture seems to decay into a similar one, whose projection into the plane of the cylinder's cross section is about the same (so they give the same longitudinal resonance) The initial textures that would be fairly consistent with the NMR-resonances are the de Gennes disgyration in the case of Saunders *et al.* and a somewhat distorted 'Pan-Am' texture (Bruinsma & Maki, private communication) in the case of Gould and Lee The deviations of the cylinder cross sections from a circular shape—they have a rounded hexagonal structure as can be seen on magnifications—probably need to be considered The only alternative mechanism that could, at least qualitatively, account for a distortion of textures in the direction of the cylinder axes in both experiments and the temperature dependence in Gould and Lee's results seem to be superfluid currents in the cylinders.

To explain quantitatively what textures are responsible for the measured NMR resonances will not be an easy but most certainly a very rewarding task. It will provide us with new understanding of textures and singularities in superfluid ^{3}He, and beyond that in condensed matter systems. □

Germs from Mars?

from Arie J. Zuckerman

THE health and environmental hazards of bringing to Earth surface soil samples from Mars, assuming that life exists on that planet, have been discussed for over a decade. On the one hand, views have been expressed that the risk of the spread of virulent Martian organisms to terrestrial life forms is so great that such an undertaking planned for the 1980s should be abandoned (see, for example Young & De Vincenzi *Science* **186**, 496, 1974) In a recent cogent discussion of the problem (*COSPAR: Life Sciences and Space Research* XVI (eds Holmquist & Strickland) Pergamon, 1978) Martin Favero points out that adequate containment facilities for microorganisms are now available, although absolute security in a scientific sense is of course impossible to guarantee It is therefore necessary to examine the risks in terms of possibilities.

Favero develops his argument for the unlikelihood of Martian microorganisms' causing harm as follows. If life exists on Mars such organisms would be adapted to low temperature fluctuations and virtually no oxygen or water in the atmosphere. It could reasonably be assumed that such organisms will be so adversely affected by the Earth's environment that Herculean scientific efforts will be required simply to maintain them. Counter arguments could be that terrestrial microorganisms survive under wide ranges of conditions, which is in principle true, and furthermore Martian organisms might remain unrecognised by the host's immune and other defence mechanisms thereby resulting in devastating infections. However, in general terms, microorganisms that have no association with man are not pathogenic, for example hydrogen bacteria, methane bacteria and blue-green algae. Another argument is that extraterrestrial microorganisms might be resistant to inactivation, for instance to heat. It is pointed out that heat which destroys carbon bonds will destroy Martian organisms If they survived, it would be because their biology was not based on carbon, in which case they should not be pathogenic for forms of life whose biology is based on a carbon system. Finally, there is the potential risk of exchange of genetic material between Martian organisms and terrestrial forms of life. However, there is no evidence of efficient transfer in nature of genetic material between heterogeneous broad groups such as bacteria, algae, fungi, protozoa, higer plants and animals, and the probability of introducing genetic material from Mars to Earth seems insignificant

This is the stuff of science fiction. Yet similarly conjectural risks had to be considered in the development of atomic energy, production of antibiotics and the use of live virus vaccines

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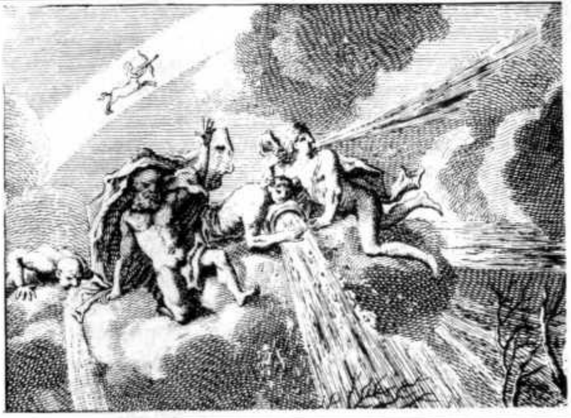

Allegory of Weather Mary Evans Picture Library

climatology supplement

The World Climate Programme

B. J. Mason

In recent years there has been an upsurge of interest in climate and its variations, the prediction of future trends and their consequences. Increasing world population and higher living standards have increased the pressure on natural resources of food, fibre, energy and water, the balance between the supply and demand for which could be seriously affected by even temporary changes in the climate of critical regions. The impact of any such changes will depend not only on their magnitude but also on the speed with which they occur. There is also increasing concern that man himself may induce inadvertent changes of climate by polluting the atmosphere with increasing quantities of carbon dioxide, heat, aerosols and chemicals. Although there has been a tendency to exaggerate the magnitude and consequences of the climatic fluctuations likely to occur in the next 100 years, future changes are likely to have greater economic and social impact than those of the past. The whole subject of climate, its variations and consequences therefore merits thorough and critical investigation.

In response, the World Meteorological Organization and the International Council of Scientific Unions, in collaboration with other international agencies, are planning a World Climate Programme spanning the two decades 1980–2000, preceded by a World Climate Conference in Geneva in February 1979 at which present knowledge will be reviewed and future plans discussed. The main objectives of the World Climate Programme may be summarised as follows: to improve knowledge of the natural variability of climate; to understand the underlying causes and mechanisms; to assess the sensitivity of climate to natural and man-made perturbations; and ultimately to predict future climatic changes; and to assist in the making of planning and investment decisions in climate-sensitive industries with a view to reducing the impact of adverse climatic changes. The Programme will have three main elements: a climate data and applications programme; a programme to investigate the impacts of climate on human activities; a programme for research on climatic change and variability.

Data and services

Although prediction of the magnitude and timing of climatic fluctuations is not yet possible, estimates of future probabilities can be made from analysis of past records of the main climatic parameters and these can serve as a useful input into the long-term planning of climate-sensitive industries such as agriculture, energy, water resources, transport and building and construction. A great deal of the available worldwide data are held in national meteorological services such as the British Meteorological Office where it is readily accessed by computer and used to answer some 30,000 enquiries each year from a wide spectrum of public services and industries. However, climatological observations need to be improved in many parts of the world where modern methods of data processing, analysis and presentation are required for the effective application of present knowledge to practical problems.

Impact studies

Very few studies have been made of the likely impact of marked and persistent changes in climate even on the major food-producing areas of the world. This would involve detailed analysis of relationships between weather and the growth and yield of the more important crops in different climatic environments. Such studies might pave the way for the forecasting of crop yields, on a regional or national basis, if and when reliable seasonal and longer term weather/climate forecasts become available.

Although it seems generally agreed that the greatest man-made potential threat to climate is the release of carbon dioxide by the burning of fossil fuels, no detailed and critical assessment of the likely effects has been made. It is not obvious that the overall result would be as detrimental as is usually assumed; it could even be beneficial. Although an average global warming of 5 °C with up to 10 °C in polar regions might well lead to substantial melting of the ice cap and widespread flooding, a more modest and more likely rise of 2–3 °C might well lead to increased food production by prolonging the growing seasons, increasing the rainfall and enhancing photosynthesis, and, furthermore, produce considerable savings in energy consumption. There will, no doubt, be some off-setting disadvantages but these should be assessed before assuming catastrophe.

Research

Study of past climates The reconstruction and study of past climates is necessary to determine the nature and extent of climatic fluctuations, to provide a guide to future changes and to validate computer simulations of past climates. Beyond the period of instrumental records the evidence rests strongly on modern techniques of radiocarbon dating and stable isotope analysis of fossils, ice cores, tree rings, and so on, which have greatly improved in recent years. These historical records, being dominated by irregular fluctuations, cannot be safely extrapolated to predict future changes. They can, however, be used to estimate the probability that fluctuations of a given magnitude, range and duration may occur within a given time interval and also to estimate the associated degrees of risk.

Climate monitoring Comprehensive data sets on the present climate are required to study mean annual and seasonal climates, for model development and validation, and to provide better climatological services. The First GARP Global Experiment (1978–79) will provide the first comprehensive set of weather observations covering one annual cycle. It will also be necessary to monitor at least the upper layers of the ocean, the ice sheets, changes in the land surface and its biological cover,

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the incoming solar radiation, and the carbon dioxide, ozone and dust content of the atmosphere. Most of these data, except subsurface ocean data, should be provided by satellites within the next few years but their processing and archiving will require considerable additional effort.

As it may not be possible to understand, much less predict, long-term climatic changes without treating the atmosphere, oceans, ice sheets, land surface and biosphere, as a single interactive system, the lack of oceanographic data may ultimately prove a serious obstacle. Although limited area and duration experiments, such as JASIN just concluded in the North Atlantic this summer, will help to elucidate interactions between the upper layers of the ocean and the atmosphere, long-term climatic changes may be induced by changes in the deep ocean circulation. These will be difficult to monitor on a fully global basis but carefully planned measurements in critical areas should provide important clues and will probably form part of the World Climate Programme to be implemented over several years as a concerted international effort.

Computer modelling Although the processes involved in the maintenance of the Earth's present climate are broadly known, those responsible for climatic fluctuations are largely unknown. Predictions of the extent and duration of climatic changes are not possible at present and must await greater knowledge and understanding of the underlying causes, whether internal or external to the atmosphere. As the climatic system embraces the atmosphere, hydrosphere, cryosphere, lithosphere and biosphere and a great variety of processes and interactions over a wide spectrum of space and time scales, their synthesis requires the building of very complex computer models of the type described in this supplement by A. Gilchrist. However, the construction, testing and evaluation of such models is a major undertaking requiring scientific resources and great computing power that is presently available only in the USA and at the British Meteorological Office where remarkable progress has been made in simulating the main features of the present global climate including seasonal changes and important regional features such as the monsoons. The models may also be used with some confidence to investigate the response of climate to conceivable natural and man-made perturbations. For example, the British Meteorological Office (Mason, B. J. *Proc. R. Soc.*, **A,** in press) has carried out a wide range of model experiments to assess the likely effects of changes in solar radiation, soil moisture and vegetation cover, sea-surface temperatures, polar ice cover, and so on, and of conceivable man-made changes in the carbon dioxide, ozone, dust and heat content of the atmosphere, and to judge whether these are likely to be distinguishable from natural climatic fluctuations. Also, by testing the sensitivity of the statistics generated by the model to variations in a particular parameter or combination of parameters, one may hope to discover the underlying causes of climatic change. However, it is sometimes difficult to assess the significance and reliability of a particular result especially if the analysis of the experiment has not clearly revealed the response mechanism of the model and there have been insufficient tests to assess the significance of the signal relative to the noise generated by the model. The response (signal) to a prescribed perturbation will be statistically significant only if the difference in the mean values of the perturbed and standard (control) states can be discriminated from the model noise as estimated, for example, by its time-averaged response to random perturbations in the initial conditions. There is a general impression that the models exhibit less variance than the real atmosphere; if this is so, it may be easier to detect a perturbation signal above the noise in a model than in the real atmosphere. It therefore seems important to compare not only mean values but the models' simulated variance with observations. All this implies a long, arduous programme of model development, improvement and assessment with the object of narrowing the degree of uncertainty in the answers they provide.

Prediction The prediction of future climatic changes, as distinct from the simulation and sensitivity studies just mentioned, poses a formidable scientific problem partly because it is not possible to verify such long-term predictions against observation. Prediction of climatic variations induced by changes in external forcing factors such as the incoming solar radiation, changes at the boundaries of the climatic system, or by man's activities presupposes that these stimuli can be predicted accurately in advance. Aside from this the precision of any climatic prediction, expressed as a set of climatological statistics, will be limited by random or spontaneous internal fluctuations within the real climatic system and by the extent to which these are reproduced by the model. When integrated over long periods with fixed boundary and external conditions models are likely to approach an equilibrium state and produce climate statistics largely independent of the transient features of the initial state.

There is, however, the possibility that the real climatic system, being highly non-linear, may be able to exist in two or more quasi-stable modes and change rather abruptly from one such state to another. The transitions between glacial and interglacial epochs are suggestive of such changes. If such transitions are externally induced, they are probably predictable, at least in principle. If, however, they can arise spontaneously from internal reconfiguration of the climatic system they may not be predictable by models that are damped sufficiently to ensure dynamical and computational stability. In any case, soundly based long-range climatic predictions are not likely to be possible within the next decade.

I hope that this introduction may give some indication of the scope, complexity and difficulty of the climatic problem, illustrate the need for substantial and sustained programmes at both national and international level, and engender a more sceptical attitude towards the ill-founded and often alarmist statements that bedevil this subject. ☐

B. J. Mason is Director-General of the Meteorological Office, Bracknell, UK.

EFFIGIES TYCHONIS BRAHE O.F.
ÆDIFICII ET INSTRUMENTORUM
ASTRONOMICORUM STRUCTORIS
A° DOMINI 1587 ÆTATIS SUÆ 40

Tycho Brahe in ſeiner Sternwarte „Uranienburg" auf der Inſel Hven
Nach einem Kupferſtich zu Tycho Brahes „Astronomiae Instauratae Mechanica" vom Jahre 1602

One of the 'earliest climatologists', Tycho Brahe (1546–1601), in his observatory on the island of Hveen (now Ven, Denmark) (taken from the 1602 edition of his *Astronomiae Instauratae Mechanica*)

Mary Evans Picture Library

Historical climatology

M. J. Ingram, D. J. Underhill & T. M. L. Wigley

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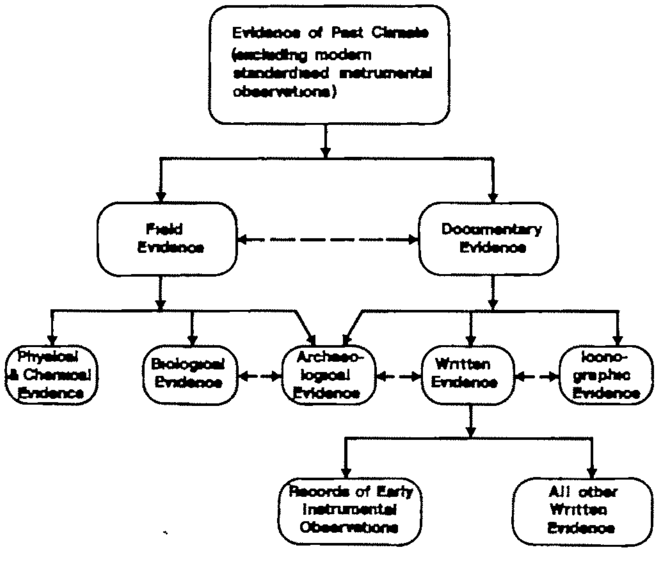
Descriptive documentary evidence is an important source of detailed information on past climates, particularly for the period between the eleventh century and the beginning of the era of instrumental meteorology. Historical climatology is concerned with the study and climatic interpretation of this evidence. However, there are many pitfalls in using historical records, associated with the fact that such records were rarely written primarily as descriptions of climate and with the fact that many readily accessible records are of doubtful reliability. Here we take a critical look at existing work in the field, with the aim of isolating many problems which need to be recognised and overcome before historical climatology can be considered to rest on a secure methodological and factual footing.

HISTORICAL CLIMATOLOGY emerged as a discipline in the late nineteenth and early twentieth century, the first major work of synthesis being by Brooks[1]. The subject's existence was at first fitful, but in the 1940s, 50s and 60s, the enthusiasm of men like Flohn, Manley, Schove, and above all, Lamb, successfully demonstrated the interest and importance of this field of study. In recent years, interest in historical climatology has grown considerably, and significant contributions have been made not only by natural scientists but also by historians (especially Ladurie[2]). This interest has arisen through an increased awareness of man's susceptibility to the vagaries of climate (the Russian wheat failure of 1972, and the Sahelian drought come readily to mind), through the realisation that climatic conditions very different from today have occurred in the recent past, and through the maxim of the acturial approach to climatic forecasting, "the past is the key to the future". In spite of this progress, no comprehensive methodology of historical climatology has been established, with the result that there exists no ready means for assessing the significance of individual contributions and, thus, of what has been achieved in the field as a whole. In view of this fact, we believe that the most useful method of reviewing the subject is to examine the problems which need to be universally recognised and overcome before historical climatology may be said to rest on a firm methodological and factual footing. In so doing, we shall as far as space permits criticise and evaluate past and current work in the field.

Fig. 1 Flow chart showing data useful for reconstructing past climates and delineating the contribution of historical climatology. The separate categories often overlap, and some of the more important interactions are shown.


Evidence of Past Climate (excluding modern standardised instrumental observations)
Field Evidence
Documentary Evidence
Physical & Chemical Evidence
Biological Evidence
Archaeo-logical Evidence
Written Evidence
Icono-graphic Evidence
Records of Early Instrumental Observations
All other Written Evidence

Scope of historical climatology

The task of reconstructing the climates of the past involves the use of many different types of evidence (Fig. 1). The successful exploitation of this material demands a varied range of skills and techniques which effectively define specialised subdisciplines of climatology. Historical climatology is best thought of as one such subdiscipline, which focuses on the study of written materials (excluding records of modern standardised instrumental observations) which bear on past climate. These materials include, not only meteorological information, but also data on such phenomena as glacier movements, phenological events and other more or less indirect indicators of climatic change.

The distinctive contribution of historical climatology ends with the advent of instrumental observations, embracing pressure and temperature measurements, which effectively began in the mid-seventeenth century. The earliest series relate to Europe. In most other parts of the world, instrumental data extend back no further than the late eighteenth century, and in many locations span less than 100 years. The earliest instrumental series, moreover, pose many problems of interpretation, and the precise dates from which they may be regarded as acceptably accurate and standardised are debatable. The elucidation of early instrumental data may be regarded as within the scope of historical climatology, but the techniques involved[3–5] are specialised ones and will not be discussed here.

Apart from early instrumental records, the written sources of climatic data are legion. They include: (1) ancient inscriptions; (2) annals, chronicles, and so on); (3) records of public administration and government, whether imperial, national, provincial or local; (4) private estate records; (5) maritime and commercial records; (6) personal papers (diaries, correspondence, and so on); (7) scientific and proto-scientific writings (including journals of non-instrumental weather observations).

How far back into the past can the historical climatologist penetrate with the aid of these records? The answer varies with location. It is impossible to give a comprehensive guide to the earliest dates from which materials exist in different areas, because much essential exploratory work to discover possible sources has not yet been carried out. For example, it seems likely that the written remains of the brilliant and complex cultures of the Middle East and India would provide rich materials for the historical climatologist, but at present they remain almost totally unexploited. However, Table 1 provides enough information to indicate the main patterns of distribution. It can be seen that in certain areas the vision of the historical climatologist is limited to only a few decades preceding the establishment of standardised instrument observations. But for considerable areas of the world there are materials extending over centuries, and in favoured locations over thousands of years.

Even in areas for which early materials survive, the distribution of sources is by no means uniform over time. For example, the European evidence for the period before about AD 1000 is very scanty. It is appreciably richer, but still very

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Table 1 Earliest dates from which written evidence of climatic phenomena survives in selected areas

Area	Earliest written evidence (approximate dates)
Egypt	3000 BC
China	2500 BC
Southern Europe	500 BC
Northern Europe	0
Japan	AD 500
Iceland	AD 1000
North America	AD 1500
South America	AD 1550
Australia	AD 1800

imperfect, for the period AD 1000–1600. Thereafter many more materials are available, but it is only in the late seventeenth and eighteenth centuries that sources become really abundant. Equally, there are great differences in quality between different types of materials, with nearly all the best sources relating to the most recent periods.

As the sources are of such variable quality and quantity, a rigorous methodology is required to reject unsuitable and inadequate material and to extract the maximum amount of information from what remains. In the following, a methodology is discussed with particular reference to data verification and analysis of information.

Data verification

Written statements about the past are not equally reliable, and indeed much material which purports to record historical events is seriously misleading. Historians have developed a sophisticated methodology for evaluating sources and rejecting unreliable information. Plainly, historical climatologists need to be similarly aware, both of the need for source criticism, and of the available critical techniques. Scientists attempting to reconstruct past climates have, in fact, often been ignorant on one or both of these counts (see, for example, criticisms in the works of Gottschalk[6-8], Alexandre[9], and Bell and Ogilvie[10]).

A critical discussion of the sources of data available to historical climatologists will reveal the significance of this fact. There exist many eighteenth-, nineteenth-, and twentieth-century compilations and syntheses of historical climatic information (the most important are listed by Bell and Ogilvie[10]). At first sight, these works seem to provide a conveniently ready-made data bank, and it is therefore not surprising that they have formed the basis of numerous attempts at climatic reconstruction. In reality many of these compilations and syntheses are highly defective as data sources, and work founded on them is liable to be inaccurate. Their deficiencies may be summarised as follows. First, their transmission of the original data is often inaccurate and incomplete. Compilations and syntheses often consist of abridged, translated, paraphrased, or condensed material. Second, many of the works under discussion include errors introduced by the author or compiler, the most common mistake being misdating. Third and most fundamentally, most compilations fail to distinguish adequately (if at all) between reliable and unreliable sources. It is true that some very recent works[6-8,11,12] are models of critical awareness. As a whole, however, the available compilations and syntheses comprise a mishmash of valuable and worthless data, and to use them uncritically for the purposes of climatic reconstruction is unacceptable. An illustration of the amount of valueless material contained in two widely used compilations is given in Table 2. In constructing this Table we have subjected the compilations of Hennig[13] and Weikinn[14] to a critical historical analysis (see below) and counted the number of items which survive this criticism. Such items are described as 'verified'.

When scientists attempt to penetrate beyond compilations and modern syntheses to the materials on which they are based, or if they seek new data, they are faced with the problem of distinguishing between reliable and unreliable sources. Various criteria may be applied to test reliability. The tests are numerous and complex, and here only the basic principles are indicated. Most fundamentally, it is necessary to examine the nature of the source to assess whether, or how far, the writer was actually interested in recording real events, for not everything which looks like history as we understand it was intended as such. Of further tests, the most important are those based on the principles of contemporaneity, propinquity, and faithful transmission. Recorded statements cannot be regarded as reliable unless it can be shown either (1) that the writer lived close in time and space to the events he purports to describe, and recorded his observations either immediately or within a very short time after these events had taken place, or (2) that he had access to first-hand oral or written reports and accurately transmitted the information derived from them.

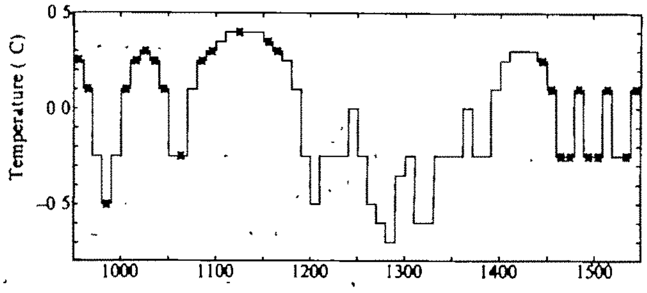


Fig. 2 Bergthórsson's original decadal estimates of temperature in Iceland showing the sparseness of data which remain after a critical examination of the historical evidence. The decades for which there is no historically acceptable information are marked with crosses (Information supplied by Astrid Ogilvie.)

Many materials, especially narrative sources such as chronicles which were often subject to constant addition and revision, emerge badly from such tests. It is frequently found that sources are in whole or in part either (1) slavishly derivative and therefore of no independent value; (2) inaccurately and corruptly derivative; (3) spurious, fabricated for some particular

Table 2 Historical reliability of two European weather compilations for the period AD 0–1000

Century (AD)	Hennig[13] Total no. of seasons described	Hennig[13] % Of seasons verified	Hennig[13] Additional information by season	Weikinn[14] Total no of seasons described	Weikinn[14] % Of seasons verified	Weikinn[14] Additional information by season	Total no of reliable seasonal descriptions available
0–99	20	50	2	8	88	5	12
100–199	15	7	2	2	50	2	3
200–299	9	0	1	1	100	—	1
300–399	16	25	3	7	86	1	7
400–499	22	14	—	2	100	1	3
500–599	44	25	5	14	71	6	16
600–699	23	13	1	3	0	4	4
700–799	31	13	6	9	56	5	10
800–899	47	57	23	35	91	18	50
900–999	45	29	6	8	75	13	19
Total	272	28	49	89	79	55	125

The additional information' refers to reliable material which we have extracted from other primary sources purpose such as to assert the rights and claims of the author's monastery; or (4) of literary, philosophical, or religious significance but of no value as records of events.

Much of the data which historical climatologists have hitherto used have been drawn, either directly or more often by way of compilations, from such unreliable sources, and are therefore subject to error. The most important errors are: (1) inaccurate or uncertain dating of particular events; (2) spurious multiplication of events through failure to recognise that accounts recorded under different dates in various sources in fact relate to a single event; (3) acceptance of accounts which are distortions or amplifications of original observations (for example, purely local events may appear through the distortions of later writers as major catastrophes covering a large area); (4) inclusion of events for which there is no reliable evidence whatsoever (such 'non-events', moreover, tend to be artificially multiplied through being assigned to different dates in subsequent derivative accounts).

These errors are not randomly distributed and are therefore not self-correcting. On the contrary, the problem of unreliable data is so great that decadal or even 50-yr series are liable to be grossly inaccurate if unverified material is used. These points may be illustrated by reference to the work of Bergthórsson[15-17] and Lamb[18,19], although it must be stressed that the studies in question were only presented as tentative preliminary analyses. Figure 2 shows the shortcomings of the early part of Bergthórsson's decadal temperature series for Iceland, itself based on the work of Thoroddsen[19]. The decades marked with a cross are those for which, as critical analysis reveals, no reliable climatic information is available. Only eight of the first 22 decades of the record have any reliable climatic information, and many of these decades have only one or two items. For the period AD 1431–1560 only one item of reliable climatic information is available. Clearly it is quite impossible to construct decadal temperature estimates for much of the period, and even 50-yr averages cannot be estimated before AD 1100 or between AD 1450 and 1550. Bergthórsson himself may have had misgivings about the decadal series shown here as it has not been published in its original form: nevertheless, it has subsequently been reproduced by other authors[15,16].

Lamb[9] has published decadal indices for England, Central Europe and the Russian Plain for the period AD 1100 onwards (discussed further in the next section). As with the Icelandic reconstruction, these indices are partly based on compilations which are now known to be unreliable, and, on this ground alone, they must be regarded as suspect: they are currently being revised in the Climatic Research Unit. Although the results of this revision are not yet complete, it is instructive to compare Lamb's indices with the work of the historians Titow[21] and Alexandre[11,12].

Fig. 3 Comparison of winter temperatures (December, January, February; °C) from three European sites for the period 1684–1738. Short-term fluctuations have been smoothed out by using a 9-yr Binomial filter. The Zürich record[30] is based partly on the observed correlation between Zürich and central England temperatures, but also on records of local snowfall and duration of snow cover. (We have filled some minor gaps in the Zürich record using the Zürich–England correlation.) Part of the central England record[31] (1707–22) is based on instrumental data from Delft.

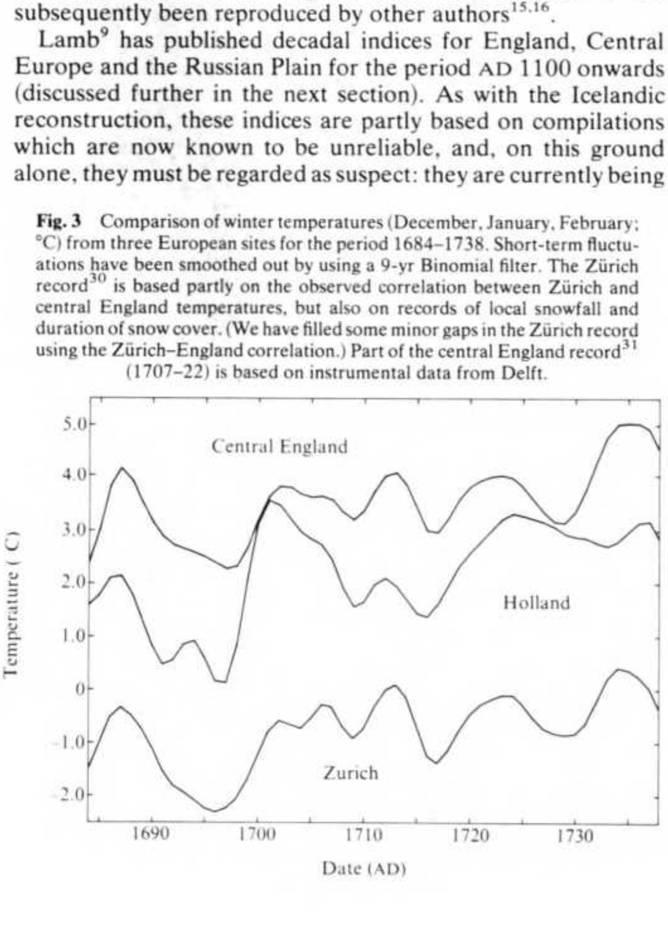


Fig. 4 A frost fair on the River Thames early in 1863.

Consider first the summer wetness indices for England for the period AD 1200–1350. Lamb (ref. 18, page 32) has compared his results for these 15 decades with primary data extracted by Titow[21] from the manorial account rolls of Winchester Cathedral. The two records are well correlated ($r = 0.77$). On the other hand, Lamb's English winter severity indices for the period AD 1100–1400 seem to be less well-founded. Here, there is no independent record for England, but there is a similar record available for Belgium constructed by Alexandre. (Alexandre[11,12] has analysed the compilation of Vanderlinden[22] in detail and found it to be seriously unreliable. In order to fill the data gaps which resulted from his source criticism, Alexandre examined a large number of primary sources which Vanderlinden had not used. From this new body of data he then calculated decadal indices using the method developed by Lamb (see below).) Comparison of Lamb's and Alexandre's indices shows only a low correlation ($r = 0.2$, compared with a correlation coefficient of 0.9 for decadal winter temperatures between AD 1750 and 1940). Although this low correlation may be partly the result of real changes in meteorological factors, it must be largely attributed to the variable, but generally poor quality of the compilations used by Lamb.

These strikingly contrasting results indicate the need for a re-examination of the data which form the basis for the currently accepted historical climate record. It is essential to build up a data bank of historical information of unquestionable reliability.

Analysis and interpretation

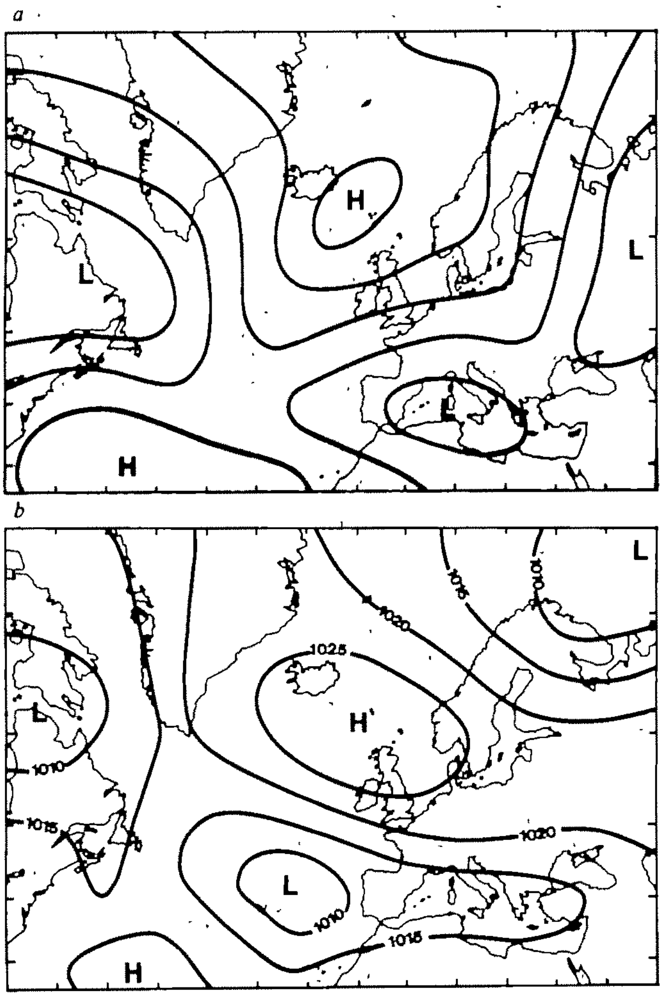
Given reliable data, the next task of the historical climatologist is to use different types of documentary evidence to produce time series which are, as far as possible, comparable with modern meteorological records. Four steps are involved: historical analysis of the material; quantification; transformation into meteorological variables; and the addition of long-term trends where necessary.

Modern instrumental observations provide the nearest approach to an objective and comprehensive record of climatic phenomena. Historical climatic data, on the other hand, embody distortions and omissions reflecting the various personal and cultural biases of past observers. The ways in which phenomena were perceived and categorised varied from period to period, place to place, and observer to observer. So also did the principles by which phenomena were selected for the record. Even the manner of recording—terse or verbose, sparsely or profusely detailed, matter-of-fact or poetically embellished—affects the information content of the record.

In the final analysis, these factors limit the quality and quantity of the information which can be derived from the documents. But they also pose problems of interpretation, in effect obscuring what information the materials do contain. This obscurity must, as far as possible, be removed by subjecting the sources to rigorous textual analysis to establish the meanings of words and categories and to determine the nature of biases. This may be achieved by reference to specialised historical knowledge of the intellectual and social milieux which gave rise to the texts, and by internal analysis of individual sources and comparison between sources relating to the same set of phenomena. Moreover, as Moodie and Catchpole[23,24] have demonstrated, the methods of analysis should be codified and systematised to ensure that individual analysts, faced with the same body of materials, will derive essentially the same information.

Although a universal set of rules cannot be defined, the analyst must ask of the verified data these basic questions: (1) Why was the information recorded? How did the purposes of the writer bias his perception and selection of phenomena? (2) What is the precise significance of such categories as 'frost', 'drought', 'flood', 'sea-ice', and so on? (3) What is the significance of terms of degree? For example, what weight should be attached to expressions like "The greatest . . . that hath been known in the memory of any man living".? (4) When did the event occur, and for how long? Changes in the calendar have occurred at different times in different places, and the precise timing of an event is not always important to the past recorder. Furthermore, the timespan covered by terms like 'summer' and 'winter' varies from record to record. (5) Where did the event occur? Did the recorder distinguish between local and 'foreign' events? The weather diary of Merle[25], kept variously in Lincolnshire or Oxford from 1337–44, is a mild example of the sort of confusion which can arise.

Fig. 5 Preliminary reconstruction of the winter 1683–84 circulation pattern (*a*), and the pressure map for January 1963 (*b*). (1683–84 reconstruction supplied by H H Lamb) Both seasons exhibited strong blocking patterns with persistent E to NE winds over England The normal winter pattern shows a belt of low pressure at the latitude of Iceland, and south-westerly winds over England, and the patterns here are highly anomalous



The next stage in analysis is to quantify the information in terms of standard meteorological variables. In general, two steps are necessary: quantification in terms of a numerical index followed by calibration of the index in terms of a variable such as temperature or precipitation. Certain types of information are already numerical in form, and if they are homogeneous and systematic they can be calibrated directly by correlation between the original record (or a modern-day equivalent) and a meteorological parameter provided there is a sufficient period of overlap between the records. One of the earliest examples of this type of analysis is the winter temperature reconstruction derived by Flohn[26] from the meteorological journals of Tycho Brahe[27] (Hven, Denmark, 1582–97) and Haller[28] (Zürich, 1546–76). Flohn found from modern instrumental observations that the ratio of days of snowfall to days of rainfall in winter was well correlated with winter temperature. Using the snow and rain records of Brahe and Haller, Flohn was able to show that winters in Zürich were about 1.3 °C colder in 1561–76 than in 1546–61; whereas the winters on Hven from 1582–97 were about 1.5 °C below those around the turn of the twentieth century. The implied severity of the latter part of the sixteenth century accords with the wind direction observations given by Brahe and analysed as early as 1876 by La Cour[27].

Similarly, De Vries[29] has calibrated data on freezing of water in Dutch canals in terms of winter temperatures to extend the record of Dutch winter temperatures back to 1634; and Pfister[30] has used data on snow falls and duration of snow cover from various historical sources to estimate winter temperatures near Zürich back to 1683. Figure 3 compares De Vries' and Pfisters' results with Manley's[31] central England temperature record (based on instrumental data) for the period 1684–1738. All three records show the striking cold of the 1690s, a decade without subsequent parallel.

Unfortunately, most historical information is not so readily quantifiable in terms of meteorological variables, as the data are generally discontinuous, non-homogeneous, and show a marked bias towards the recording of extreme events. In such cases the documentary evidence must first be transformed into a numerical index. One of the first to do this was Brooks[1] who derived 50-yr indices of wetness and winter severity for Europe using data from a number of compilations. Lamb[19] refined this work by calculating decadal indices for Europe near 50°N at three longitudes, 0°E (England), 25°E (Central Europe) and 50°E (the Russian Plain), back to AD 1100. Lamb's indices may be defined as follows. For 'winter severity', the index is $C-M$, where C is the number of unmistakably cold winter months in a decade and M the number of unmistakably mild months; and for 'summer wetness', $W-D$, where W is the number of summer months with evidence of frequent rains and D is the number of months with evidence of drought per decade.

One of the underlying assumptions of indexing, and the basis for expecting indices to reflect the meteorological variables, temperature and precipitation, is that there are few, if any, severe seasons for which we have no written record. Previous historical climatologists have tended to believe that there are few such data gaps, mainly because they had no way of knowing the poor quality of their sources. We now know that there are gaps in the record, especially before AD 1500, and that early impressions were false because of the amount of duplicated, fabricated and erroneous information in the widely used compilations.

This raises a question: how many gaps are permissible before decadal index values become unrepresentative? Different types of index show differing sensitivity to missing data. 'Difference' indices, as used by Lamb, are quite sensitive, whereas 'ratio' indices show less sensitivity. (An example of a ratio index for winter severity is $(C-M)/(C+M)$ where C and M are the

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number of cold and mild months, respectively.) The sensitivity of indices can be tested by a Monte Carlo type of simulation where one starts with an artificially constructed (but realistic) complete record and progressively introduces more and more random gaps until there is a significant change in the average index value for an ensemble of decades. Such simulations show that ratio indices can tolerate small gaps in the record. (Equally important, they indicate that ratio indices are robust to uncertainties in the perception and/or actual frequency of extremes.)

The second step in quantification is to convert from index to meteorological parameter. Here the method is to correlate the index values with instrumental temperature or precipitation data over an overlap period. If a significant correlation cannot be established then the index itself is of doubtful value, but if there is a correlation then it can be used to interpret index values before the overlap period directly in terms of meteorological variables. Lamb[32] found that the correlation coefficient between summer wetness and late summer (July plus August) precipitation was 0.91, and the correlation between winter severity and mean winter temperature (December, January, February) was −0.87. However, neither index shows any significant correlation with summer temperatures. We note again that extrapolation back in time, even using the above high correlations, is suspect because, whereas the index values are reliable for the correlation period, their reliability is increasingly uncertain earlier in the record.

Detail from *A Winter Scene with Skaters near a Castle* (about 1609) by Hendrick Avercamp (Netherlands)

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The third step in producing meteorological time series from historical data is to introduce long-term fluctuations. It has already been noted that historical data (excluding the most easily quantifiable items discussed initially) tend to be biased towards the reporting of extreme events. But 'extreme' here is not absolute, and is only relative to the observer's own idea of what is normal, which in turn is largely determined by his own experience. Long-term trends in the norm go unobserved, particularly because they are small relative to year to year fluctuations. As a crude meteorological instrument, man tends to act as a high pass filter and his subjective observations show only short-term fluctuations about an ever-changing norm. The response of this filter is uncertain: for example, a trend may show up as a series of extreme years giving the appearance of a step-function change in climate.

Detail from *The Castle of Muiden in Winter* (1658) by Van Beerstraaten (Netherlands)

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This problem has long been realised by climatologists (see, for example, Brooks (ref. 1 pp. 329–330) and the solution suggested by Brooks still applies. This is to use either proxy data, or records which are not subject to filtering, to derive long-term trends independently, and to use these as the background for shorter-term fluctuations. Glacier fluctuations, pollen records, lake levels, tree line changes, vegetation limits and others are suitable. Some of these items may themselves derive from historical documents and so require care in compilation and analysis, whereas others are imperfectly understood as climatic proxies and can only be used after detailed individual studies of their responses to climate.

A more ambitious use of historical data is to construct a time series of maps of seasonal circulation patterns based on qualitative data from different locations. Such maps provide a synthesis of the diverse types of data which are available for Europe and the North Atlantic. Starting with a well distributed set of climatic descriptions pertaining to a particular season (or month), putative isobars can be drawn so that the implied wind pattern and the positions of centres of cyclonic and anticyclonic activity are consistent with the descriptive evidence. The principle involved is the same as that used by twentieth-century meteorologists to extend the surface pressure patterns on synoptic maps outside the region for which pressure data are available. Wind direction data are particularly useful for this type of analysis. The results depend on the experience of the analyst whose performance can be tested by using descriptive material from times when instrumental pressure maps are available. An example of this work is shown in Fig. 5, a reconstruction for the severe winter of 1683–84 (see also Fig. 4), arguably the most severe winter in England since 1659, and one of the most severe for Europe as a whole (possibly surpassed by 1708–09; ref. 33). The pressure map for January 1963 is shown for comparison: the similarity is

Dutch winter scene (seventeenth century engraving)

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striking and reinforces earlier comments by Manley[34] on these two winters.

Using the reliable historical information which is now available, it should be possible to reconstruct circulation maps for many of the extreme seasons in Europe back to at least AD 1500, covering the worst period of the 'Little Ice Age'. The Little Ice Age is a period which is unique in the record of the past 3,000 years at least[35]. Most importantly, it has no equal in the period of instrumental meteorology, and may provide information on 'no analogue' situations which might recur in the future. But is this period just one where extreme events similar to those of today occurred more frequently; or were the extremes of the seventeenth century in some way fundamentally different from those of today? This type of analysis may help us to answer this question.

Conclusions

We have tried here to show a few of the shortcomings of existing research in historical climatology, tempered by some of its successes, and its potential for the future. The main defect in previous work is that much of it can now be seen to have been based on the sands of unreliable data. We have demonstrated the significance of this in two ways: by analysing some of the commonly used sources to find out the extent of unreliability (see Table 2), and by comparing some accepted reconstructions with more historically acceptable analyses. We believe that, at the very least, the details of shorter time scale fluctuations are subject to considerable doubt, and that revisions are essential. Nevertheless, although many of the studies published to date are open to criticism, we would emphasise that their authors were the pioneers in a developing science, whose efforts have aroused scientific and public interest. The most appropriate way of appreciating their work is to develop more rigorous methods through the application of established historical techniques and the refinement of current methods of quantification. We believe that this will result in a significant improvement in man's knowledge of past climates.

We thank Astrid Ogilvie for access to her verified data for Iceland, and H. H. Lamb for the 1683–84 reconstruction used in Fig. 5. This work was supported by grants from the Rockefeller Foundation (M. J. I. and T. M. L. W.) and the US Department of Commerce (D. J. Underhill), the latter through the US National Oceanic and Atmospheric Administration. We thank J. Craddock, H. H. Lamb and G. Manley for useful discussions.

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Tree-ring evidence of past climatic variability

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The increasingly visible impact of climatic variability on human affairs lends a sense of urgency to the task of better understanding the workings of the Earth's climatic system. Actual instrumental observations of climate are relatively short, and we must therefore turn to other sources for information about past climates to help develop and test the models that may enable us to predict climatic anomalies such as prolonged droughts or a series of severe winters. Tree-rings are one of the best sources of climatic proxy information. They can provide long, accurately dated, year-by-year records at many points around the globe, and bridge the gap between recent instrumental or historical data and the longer but more generalised geological records. Variations in the width of annual rings reflect the influence of climatic factors that limit the biological processes governing ring formation within a tree. Study of reconstructions of long records of a variety of climatic and related variables, such as temperature, precipitation, stream runoff and barometric pressure over periods of several hundred to several thousand years strongly suggests that the climate of the past century or so is not representative of the conditions that have frequently prevailed over long periods. Proxy records are thus a great help in our efforts to anticipate or predict future climate, which may be significantly different from the recent climatic past.

GREATER understanding of the global climatic system has become an increasingly important goal of scientific endeavour in a world that is faced with an expanding population and increasing pressure on energy, water and food resources. Growing awareness of the problem of climatic variability and its impact on human affairs is demonstrated by a series of recent major reports by the US National Academy of Sciences[1–3], the Australian Academy of Science[4], the Science Council of

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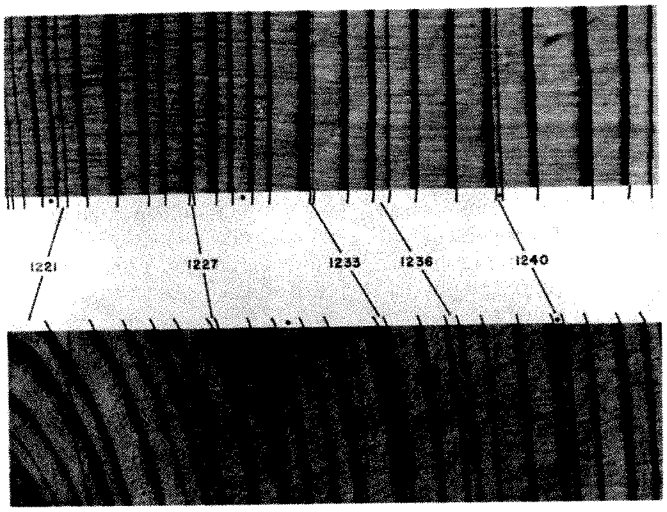


Fig. 1 Climatic sensitivity and crossdating. Douglas fir (*Pseudotsuga menziesii*) beam from a late thirteenth century Indian ruin in northern Arizona shows distinctive sequences of wide and narrow rings reflecting rainfall variations. The narrow rings represent years that were drier than normal.

Canada[5], and by other national and international agencies.

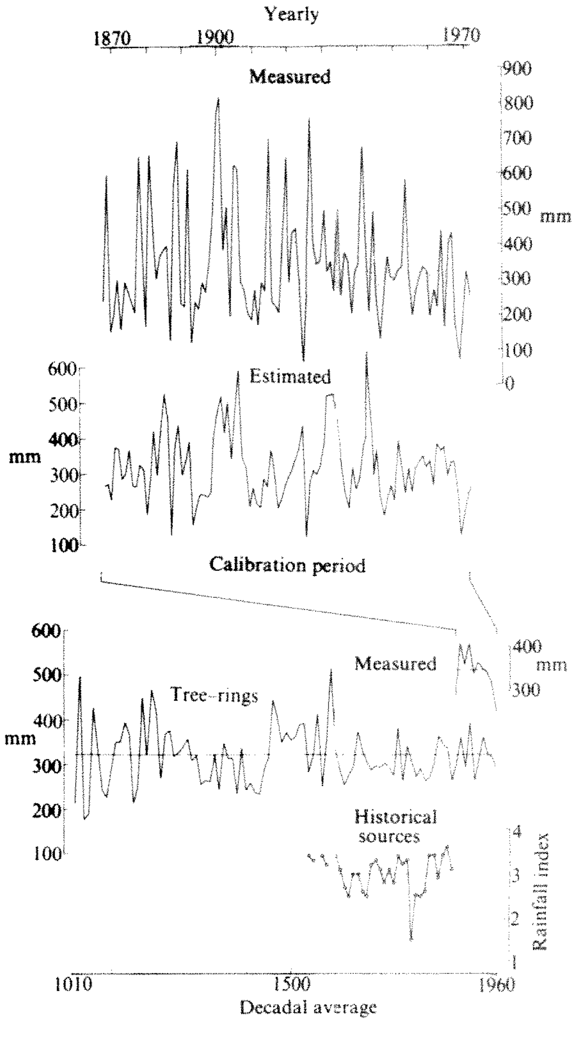
The basic problem is that we do not know enough about the workings of the climatic system, which includes the oceans, land areas, and ice sheets as well as the atmosphere. Because of its complexity, predictions about the future behaviour of the system must ultimately come from physically based numerical models which embody our understanding of the processes involved. However, the existing observational data base may be inadequate for the development and verification of models capable of accurate climatic forecasts. One of the most serious constraints that could emerge is that a model 'tuned' to generate the present climate with adequate precision may not be able to reproduce climatic states that have existed in the geologically recent past, and that could recur in the immediate future. An important step in the verification of a model (with important implications for its use as a forecasting tool) would be to test whether it can generate not only plausible mean conditions, but also time series with variance spectra similar to those of data from the real world. Observational, historical and climatic proxy data for time periods independent of those used in development are thus needed for complete model verification.

Palaeoclimatic records

The records of actual instrumental observations of climate are inadequate for study of climatic variations on time scales of decades to millennia[1]. Continuous records of surface elements such as atmospheric pressure, temperature and precipitation go back 300 yr at most, and then in only a few localities in the Northern Hemisphere. In most areas, reasonably complete surface coverage exists for only the past 50–100 yr. Upper air soundings have been regularly made on a global basis for only the past two or three decades, and satellite observations are an even more recent innovation. Fortunately, information about climate before the beginning of instrumental observations can be obtained from study of a wide variety of physical and biological phenomena that respond in some way to climate. The "unique value of proxy data for studies of climatic change" has been recognised by the US Committee for the Global Atmospheric Research Program[1]. Among its principal recommendations is a programme for the preparation and maintenance of climatic data banks, and of analyses based on both current and palaeoclimatic data. It envisages the use of palaeoclimatic records for setting boundary conditions and for model verification and also views these records as our only documentation of the extreme states of which the Earth's climatic system is capable. The WMO-ICSU Joint Organising Committee[6] also states that "special efforts should be made to locate, make available, and analyze . . . a wide variety of human recordings and natural indicators of climatic conditions in earlier centuries and millennia . . .". Here, the emphasis is on the use of time-series analysis of proxy records to improve understanding of physical processes and to explore the possibilities of empirical prediction, as well as on verification of numerical models. According to the Geophysics Research Board[2], the design of more robust water-resource systems would be helped by palaeoclimatic research which "offers a promising approach to better estimates of past climatic variability and drought probabilities." Palaeoclimatic data thus promise to play an important part in further development of climate theory as well as in more immediate practical applications.

Because climate varies over a large range of time and space scales, it is important to identify the time periods and geographical areas in which data would be most critical in helping to understand the existing climate. The Earth is now, and has been for some 10,000 yr, in an interglacial climatic state, characterised by relatively high sea levels, relative warmth, and largely ice-free conditions in temperate latitudes. However, even the present interglacial can be subdivided into periods of distinctively different climate. The last globally significant change seems to have taken place about 3,000 yr ago. Widespread cooling and associated circulation changes at this time are fairly well documented in the Northern Hemisphere. Smaller but at least locally significant variations have occurred in more recent

Fig. 2 Rainfall estimation from tree-ring data. Total annual precipitation at Santiago, Chile, is calibrated with ring-width indices at a nearby site using simple linear regression methods (correlation coefficient 0.65). This relationship is first used to estimate rainfall for the calibration period of actual meteorological observations (above), and then for the total length of the tree-ring record (below). The dendroclimatic reconstruction compares fairly well with semiquantitative information from historical sources as given by Lamb[30].



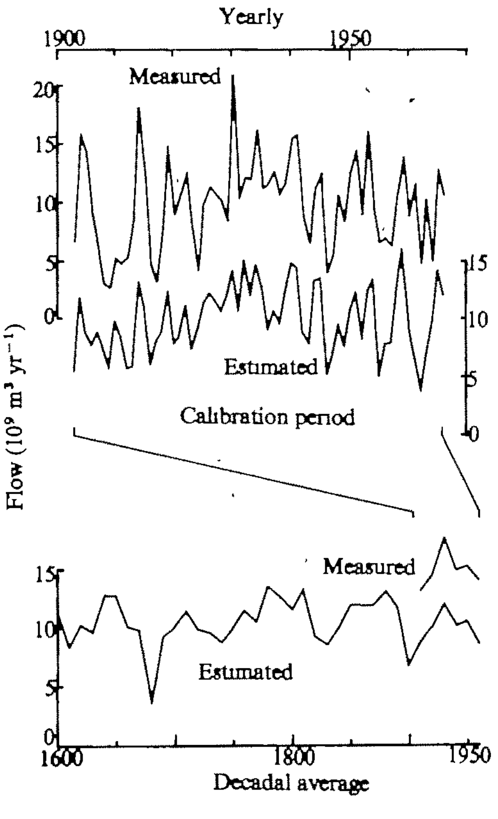
times. As pointed out by Kutzbach[7], palaeoclimatic records of the past several thousand years thus seem most relevant to study of the natural variability of our present climate, and fall within the scope of current planning. The kinds of natural variations we can expect in the near future will very probably be similar to those experienced in the relatively recent past.

Tree rings as climatic proxy data

Within this time framework, the annual rings of trees are one of the most valuable sources of climatic proxy data. Trees are found in most land areas of the Earth, they can reach ages of several hundred to a few thousand years, and under favourable circumstances their growth rings can be dated to the exact year of formation. Dendrochronology can provide long, continuous sequences of accurately dated annual rings, and is a dating tool that has found applications in fields ranging from archaeology to vulcanology. More important here is the potential for detailed palaeoclimatic reconstructions, because the physical and chemical properties of the annual rings can yield records of a broad range of environmental variables that are directly or indirectly linked to climate. Tree rings combine environmental 'sensitivity' and great length of record with the potential for resolution to individual calendar years, or indeed, to individual seasons. Thus, tree-ring-based proxy data series bridge the critical gap between the longer but more generalised geological records of climate, and the shorter and more detailed records of instrumental observations of climatic, geophysical, geochemical and astronomical variables that are critical to understanding the causes of climatic variability and change.

Dendroclimatology, concerned with the reconstruction of past climates through study of the properties of tree rings, has developed rapidly within the past 15 yr. Major factors have been the greatly increased speed of data processing possible using modern computers together with the development and application of powerful multivariate statistical techniques, greater understanding of the biological processes linking climate and tree growth, and the expansion of applications to broad new geographical areas. Exciting results are beginning to emerge from the use of a variety of new methods now being developed for investigation of the physical and chemical properties of the wood itself, such as stable isotopic composition[8-14], density[15,16] and amino acid composition[17]. However, although a large body of understanding and expertise has evolved for dealing with the climatic interpretation of ring-width variability, more recent techniques based on physicochemical properties of tree rings are still in their infancy. Moreover, study of the traditional dendroclimatic variable—the width of the annual ring—has yielded a large body of palaeoclimatic data that serves to illustrate the range of possible applications, the nature of long climatic proxy series, and the implications of past climatic variability for the future.

Fig. 3 Streamflow reconstruction. Tree-ring records from semi-arid sites in the Neuquén River basin in Argentina were incorporated in a canonical regression analysis[35] to estimate annual runoff at Paso de los Indios for the calibration period of gauging station record (above) and for the entire period 1600–1967 (below). Trees and drainage basins integrate fluctuations in temperature and precipitation in similar fashion, as shown by the good correspondence of decadal average values, and a correlation coefficient of 0.73 between measured and estimated annual values.



Ring-width variability

Many of the biological insights, recent technical developments, and palaeoclimatic applications of ring-width data have come through the work of H. C. Fritts and his associates at this laboratory. His book[18] gives a thorough review of the field, develops a methodology, and illustrates its use, primarily through examples from western North America. A good, botanically orientated review of tree rings as a natural data storage system has been published[19] in Great Britain with a lengthy bibliography, and the potential for application of dendroclimatology there is illustrated by recent findings[20-22]. There is also renewed interest in climatic and other applications of tree rings elsewhere in Europe[23,24] and in eastern North America[25], and a major effort is underway in the temperate latitude Southern Hemisphere[26], including Australia[27] and New Zealand[28].

The basic premise underlying the interpretation of ring-width variations in climatic terms is that climatic factors can directly or indirectly limit growth processes in trees[18]. The most suitable trees (with climatically 'sensitive' ring series) are often found in marginal situations, such as at the northern treeline, at the upper treeline in the mountains of temperate latitudes, near the arid forest border in desert regions, or as suppressed understory trees in temperate rain forests. Under such circumstances, in an adverse year, one or more environmental factors, such as soil moisture content, air temperature, or sunlight may frequently limit biological processes such as photosynthesis that are essential for development of an annual ring of normal width. Because all the trees in the same area experience the same general weather conditions, all those in appropriate ecological situations will form a narrower-than-usual ring in a bad year. The resulting synchronous sequences of wide and narrow rings provide the basis for cross-dating of tree-ring series (Fig. 1) as well as for palaeoclimatic inferences.

Data collection

A variety of approaches can be followed in extracting climatic proxy information from tree rings. A general procedure is first to select field collection areas and to identify suitable species, and then obtain tree-ring samples in the form of increment cores and cross-sections. Experience indicates that samples for ring-width analysis should include cores from 10 to 30 trees on each geographically limited and ecologically homogeneous site. After sample preparation, cross-dating of the tree-ring series (Fig. 1) leads to development of a site chronology, ensuring accurate dating of the annual rings. Measurement of ring-widths of selected samples follows. Transformations are then applied to individual radial series of ring-width measurements to remove biological trends. The next step is internal evaluation of the dendroclimatic potential of the site, based on the degree of common variation of tree-ring properties through time. Results of cross-correlation tests and analysis of variance, for example, indicate the degree of correlation between radii on the same tree, and between trees on the same site, and are measures of the

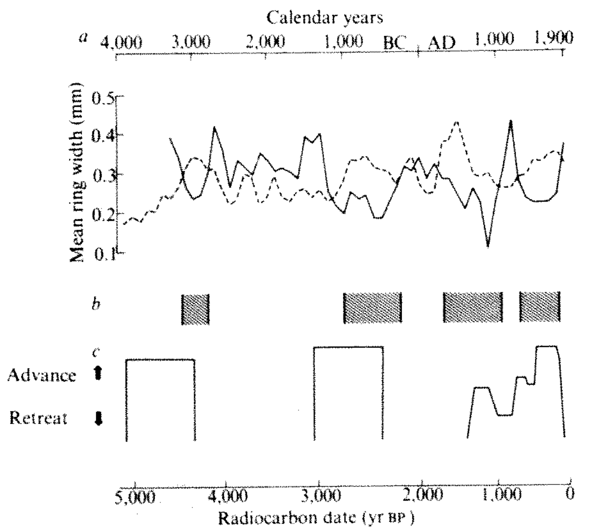


Fig. 4 Long-term fluctuations in temperature and precipitation. Although not explicitly calibrated in terms of climatic parameters, records from bristlecone pine in the White Mountains, California, offer strong evidence for changes in climate spanning centuries to millennia. (*a*), Successive 100-yr means of the average annual ring-width in large samples of trees from two ecologically distinct habitats, with quite different ring growth responses to climate. ——, Upper treeline (temperature-sensitive); ······, lower forest border (drought-sensitive). Inferred periods of relatively cool and moist climate in the White Mountains (*b*) agree well with evidence (*c*) of periods of growth and extension of mountain glaciers, mainly in western North America and northern Europe. Preliminary data for the lower forest border from C. W. Ferguson (personal communication); upper treeline curve from ref. 36; Holocene glacial fluctuations from ref. 37.

common climatic signal present in the data. Finally, a mean value function is computed by averaging annual values of ring-width indices to produce the final index chronology for the site.

The kind and amount of climatic 'information' contained in a tree-ring data series for a particular site can be established by comparison with nearby weather records. Multivariate techniques have been used to develop a transfer function (response function) relating departures of total weekly, monthly, or seasonal precipitation and mean temperature to departures in the tree-ring variable of interest[29]. The weather data may be from individual station records or may represent regional averages. The nature of the response function will, to some extent, determine the kinds of palaeoclimatic reconstruction that can be attempted using a particular tree-ring data series or set of series. It should be emphasised, however, that a diversity in response functions for tree-ring data from a given region is often of real advantage for palaeoclimatic inference. This kind of diversity stems from differences in response between species, and between trees of the same species in different habitats. Thus, an effort is made to maximise the information contained in a regional tree-ring data array by sampling trees on sites representing a range of altitudes and microclimatic situations, and to develop tree-ring chronologies for trees of different species in the same areas. Possible differences in the trees' response, both to different climatic variables and to the same variables in different seasons of the year, can be tested by multivariate response function techniques. The results help in determining the kind of approach to be used in palaeoclimatic reconstruction.

Palaeoclimatic reconstruction

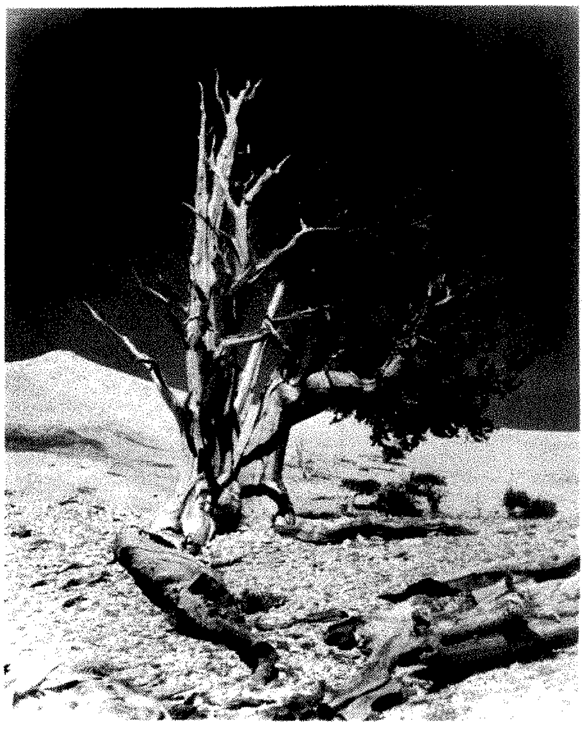
Several kinds of palaeoclimatic indices can be derived from tree-ring data. The kinds of climatic variables analysed, the spatial scale of the analysis, the degree of resolution in space and time, and the accuracy of the reconstruction or estimation will depend mainly on the inherent properties of the tree rings available for study and on the location of the trees. The proximity, quality and length of the available climatic records may also place some constraints on the scope of analysis. The quality of the proxy climatic records obtained from tree-ring data is probably best illustrated by examples of recent (and in some cases, preliminary) findings that range from estimates of a single variable at one point, based on a single tree-ring chronology, to the reconstruction of large-scale spatial anomaly patterns of several climatic parameters derived from analysis of data from a large network of tree-ring stations.

The simplest approach to palaeoclimatic reconstruction with tree-ring data is to use a highly stratified sample from trees responding (to a first approximation) in a simple manner to a single dominant climatic variable, such as precipitation. In this way, one can estimate past precipitation amounts at a point, along a meridional or zonal transect, or over a region. This kind of application is illustrated by a series of estimated annual precipitation amounts since AD 1010 for Santiago, Chile (Fig. 2). Inspection of this proxy record suggests that long periods of above- or below-average rainfall have occurred in the historically recent past, but are not well represented in the brief observational record. The ultimate extension of the single-variable approach is to use a regional array of tree-ring data points to infer past spatial anomalies in some dominant climatic parameter. This has been done successfully for precipitation[31,32] and for temperature[33] in the western US.

Complex, as well as simple, climatic variables can be reconstructed from tree-ring data. For example, stream runoff is often found to be positively related to precipitation, but negatively related to temperature, because warmer-than-usual conditions increase evapotranspirative water losses within the drainage basin. High temperatures can also limit growth processes in drought-sensitive trees directly as well as through depletion of soil moisture. Thus, tree-ring records can yield good proxy records of streamflow[34] as illustrated in Fig. 3, which shows large long-term fluctuations in flow from a major river basin in Argentina.

Past records of two variables can also be inferred at a single point, yielding information of great value for synoptic scale diagnostic analysis[36]. This is illustrated by comparison of very long records from bristlecone pine (*Pinus longaeva*) in the White Mountains of California (Fig. 4). In their lower altitudinal range,

Fig. 5 Biological impact of climatic variability. Bristlecone pines at upper treeline in the White Mountains of California are sensitive to fluctuations in warm-season temperatures. In a cool period (AD 1300–1850) coincident with Europe's historic 'Little Ice Age', the average growth rate dropped to half its former value (Fig. 4) and many trees succumbed to adverse growing conditions[38], leaving the trunks littering the foreground.



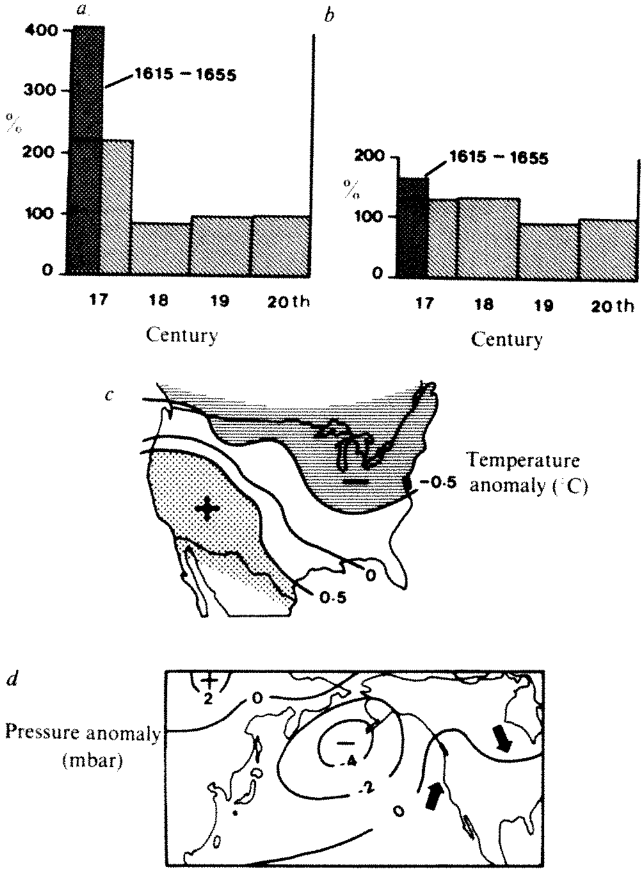


Fig. 6 Past climatic anomalies. Data from a grid of tree-ring sites in western North America have been used to reconstruct surface pressure anomaly patterns over the subhemisphere centred on the North Pacific Ocean for each season of each year, 1602–1962. Correlation analysis permits identification of the past occurrence of winters with pressure anomaly patterns like those of 1976–77 (*a*) and 1977–78 (*b*) over the past four centuries. Their apparent frequencies (above) are expressed as a percentage of their frequency in the twentieth century. Winters like these were especially common in the period 1615–1655. Reconstructed temperature (*c*) and pressure anomaly patterns (*d*) (based on 1901–1970 normal period) are shown below. Arrows indicate anomalous flow directions, bringing cold air to eastern North America. Adapted from Fritts and Lofgren[40].

ring-width is limited by drought conditions, especially low precipitation, whereas at upper treeline (Fig. 5) growth is mainly limited by low temperatures. Therefore, it is possible, by taking advantage of this diversity in climatic responses, to more fully characterise the nature of past climatic variations in this area. The warmth and humidity of the past century seem atypical in the light of the longer record.

Although ring-growth in trees can sometimes be approximated as a function of a single dominant climatic variable, a complex response to several environmental variables that are linked to climate is the more general case. Furthermore, the response to each variable, such as temperature, may change in magnitude or sign depending on the season of the year. For example, higher-than-usual temperatures in the previous autumn may be related to formation of a wider-than-usual ring in the current growing season, whereas high temperatures in the period immediately before and concurrent with the growing season may favour formation of a relatively narrow ring. Such relationships have been determined, and are readily interpretable in terms of the biological processes governing ring-growth[18]. Although comparatively little is yet known about the detailed relationships between climate and densitometric and isotopic variables, similar complex relationships are likely to be found in many cases[14].

Multivariate statistical methods have been successfuly applied to the simultaneous estimation or reconstruction of large scale spatial anomaly patterns of individual climatic variables, using tree-ring data representing a diversity of complex climatic response functions[29]. Techniques such as principal component analysis, stepwise multiple linear regression, and canonical regression analysis are used as tools for palaeoclimatic reconstruction on local to subhemispheric scales. Fritts *et al.* have been successful in using tree-ring data from western North America to estimate past sea-level pressure anomalies over a large area of the Northern Hemisphere[39]. In recent work, they evaluated the unusually cold (in the eastern US) winters of 1976–77 and 1977–78 in the perspective of several hundred years of proxy climatic record provided by tree rings. Their findings, shown in part in Fig. 6, strongly indicate that severe winters similar to these have occurred with great frequency at certain times in the relatively recent past. During part of Europe's historic 'Little Ice Age', the period 1615–1655, 70% of the winters seem to have been characterised by circulation patterns and associated temperatures over North America similar to those of one or the other of the past two winters.

Future directions

The future directions of dendroclimatic research will undoubtedly be strongly influenced by the needs of the climatological research community for specific kinds of proxy data in particular geographical areas. Nonetheless, present trends should lead to further expansion of the geographical data base, increasing contributions of new kinds of palaeoclimatic information from physicochemical analyses, and more intensive investigation of the time-series properties of tree-ring and derived data. The need to improve our ability to anticipate or predict future climate may result in the greater use of dendroclimatic data for actuarial-type analyses of the probability of future recurrence of past climatic episodes, and perhaps even for empirical forecasting.

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Mechanisms and models of climatic change

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Many theories about climate change are essentially untestable, but we can still develop a consistent model based on understandable physics—in fact the data cannot be interpreted without one. Shutts and Green believe that there is some fundamental defect in all present models: it could be something physically improbable, like an unreasonable effect of tiny solar variations. More likely there is a fundamental lack in our appreciation of how very interactive systems behave.

CLIMATE modellers, in attempting to understand climatic an infinite number of mathematical models of physical processes and feedback mechanisms which are believed to be important. Economy of representation is achieved by the parameterisation of many physical processes. For instance, nearly all current models have avoided solving the equations for radiative energy transfer through the atmosphere and have made do with simple empirical schemes. Even though the theory of radiative transfer in the atmosphere is well known, its computation is tedious and parameterisation is simple and effective. Such an approach assumed that an assemblage of empirical processes (each one realistic in isolation) will behave like the real atmosphere, which has an infinite number of degrees of freedom and connections between processes. The art of parameterisation requires a blending of theoretical understanding, observation and physical intuition to achieve this and is a necessary part of modelling most, but especially very complicated, physical systems.

Definitions of climate

Is any useful purpose served by trying to invent a universal definition of climate? From a philosophical point of view, a definition in physics has little value outside the context of a mathematical theory. For example, the concept of heat becomes hopelessly ambiguous and devoid of use without the first law of thermodynamics, even though it retains its intuitive association with fire and physical sensation. Synoptic meteorologists go to some lengths to define 'blocking': a condition observed on maps of pressure patterns intimately related to anomalous weather conditions. Such people are unanimous in their diagnosis of a 'blocking situation' but are hard pressed to agree on a definition.

Over 20,000 years ago, nearly half the Earth was covered with ice to a depth of 1 km. There was a time a few hundred years ago when 'Frost Fairs' used to be held on the Thames River in London, and North West Europe suffered a drought between 1975–76. This is sufficient information to convey the subjective idea of climate change.

Climate is conventionally regarded as the state of the atmosphere averaged over an appropriate interval of time. If we believe that the climate changes in response to changes in 'external' processes, then the time interval can be chosen so that they vary little over its duration. By external processes we would certainly include variations of the intensity of solar radiation, the orography and perhaps also the state of the deep ocean and cryosphere. These definitions imply that the atmosphere is in a state of near-statistical equilibrium and responds to slow changes in the external factors.

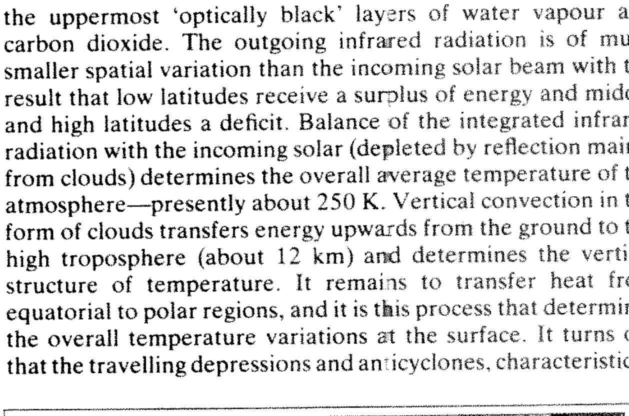
The concept of quasi-static changes is a familiar feature of thermodynamics, and it is not surprising that many of the methods of statistical physics are 'borrowed' by the climatologist. Climate is sometimes thought of as an average over a hypothetical ensemble of atmospheric states that are in equilibrium with the slowly changing external factors. But, for instance, if the atmosphere always existed in one of two radically different states (glacial and non-glacial perhaps) this definition would hardly be appropriate. It would be much more useful to describe such a climate as the probability of the two states.

But what if climate changes are completely 'internal' and occur with fixed solar radiation, orography and ocean state (that is, are merely internal 'thermodynamic' fluctuations)? Choice of an averaging time interval is then more arbitrary, but at least should be sufficiently long to filter out 'weather' fluctuations. Definition of climate and its subdivision from medium-range weather should be left to the philosophers.

Hypotheses of climate change

Before launching into an account of the possible causes of climatic change, we will briefly describe the principle aspects of the general circulation of the atmosphere and its cause.

The atmosphere behaves as a heat engine in response to its nonuniform absorption of energy radiated from the Sun. The resulting spatial variation of internal and gravitational potential energy is converted into kinetic energy of air motion by processes such as ordinary convection. The distribution and intensity of solar radiation is controlled primarily by the geometry of the Earth and its orbit. Much of the incident radiation (about 30%) is reflected back to space by clouds, ice and the type of land surface. The atmosphere is quite transparent to this short wavelength solar radiation, with most of the energy occurring in the visible part of the spectrum. Energy balance is achieved by terrestrial re-radiation (at infrared wavelengths) to space from the uppermost 'optically black' layers of water vapour and carbon dioxide. The outgoing infrared radiation is of much smaller spatial variation than the incoming solar beam with the result that low latitudes receive a surplus of energy and middle and high latitudes a deficit. Balance of the integrated infrared radiation with the incoming solar (depleted by reflection mainly from clouds) determines the overall average temperature of the atmosphere—presently about 250 K. Vertical convection in the form of clouds transfers energy upwards from the ground to the high troposphere (about 12 km) and determines the vertical structure of temperature. It remains to transfer heat from equatorial to polar regions, and it is this process that determines the overall temperature variations at the surface. It turns out that the travelling depressions and anticyclones, characteristic of


Frost Fair on River Thames, 1814

Mary Evans Picture Library

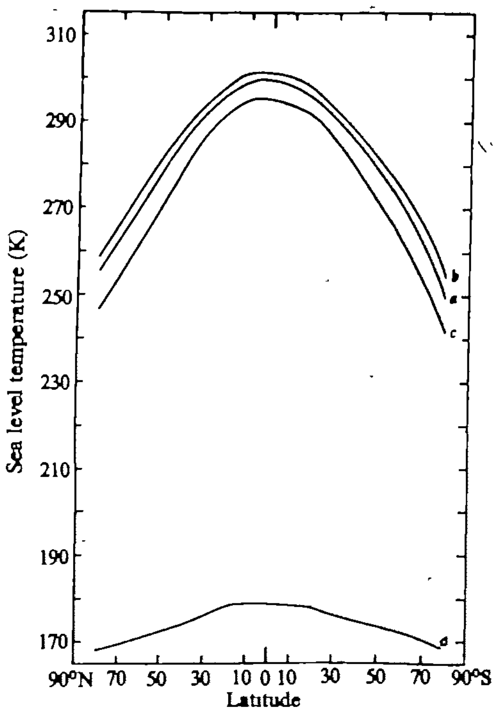


Fig. 1 Steady-state temperature distributions for various values of solar input and parameterisation[33] showing the calculated extreme sensitivity of surface temperature to small variations in solar input. Global mean temperature (K): *a*, 287.06, *b*, 289.81, *c*, 281.22; *d*, 174.65. Solar input. *a*, no change; *b*, +1%; *c*, −1%; *d*, <1.6%

unsettled weather, are the primary agency for transporting heat polewards in the atmosphere. These large scale eddies are dominated by effects due to the Earth's rotation and tend to redistribute angular momentum, causing the predominance of surface westerlies in middle latitudes, and easterlies elsewhere. These wind belts in turn determine the climatic belts of the planet.

Mountain ranges and heat anomalies, like warm winter oceans, tend to obstruct the circulating air, thereby creating stationary wave motion. The resulting nearly steady motion accounts for much of the zonal asymmetry of the climate. The Asiatic winter anticyclone and summer monsoonal low pressure are two outstanding examples of this type of local climatic variation.

(1) Solar energy output changes: The most obvious hypothesis for climate change attributes fluctuations in the global mean temperature to variations in the energy output of the Sun. Observations only in the past few decades have barely been able to detect changes in the solar irradiance of the order of 0.1% over 27 days. Several investigators[1,2] have correlated periods of high solar activity (indicated by the presence of sunspots) with anomalous tropospheric weather events such as 'blocking' or thunderstorm frequency.

The infamous 11-year sunspot cycle has been found to be astonishingly well correlated with a variety of atmospheric parameters (for example, hot summers, rainfall, lightning frequency) but in the absence of a plausible physical theory to account for them, scepticism remains.

(2) Orbital changes affecting solar insolation: Palaeoclimatological records for the last million years (the Pleistocene period) and particularly estimates of the global ice volume changes by analysis of the isotopic oxygen content of fossil plankton, indicate a strong periodicity of 100,000 years. Milankovitch[3] proposed that the secular variations of the Earth's orbit about the Sun would be sufficient to give fluctuations in the solar constant of a few per cent.

These orbital changes are due to changes in (i) the eccentricity of the orbit with a period of 96,000 yr; (ii) the obliquity of the Earth's axis with a period of 40,000 yr; (iii) the Earth's tilt by precession, with a period of 26,000 yr; and (iv) the orientation of the elliptical orbit which rotates with a period of 96,000 yr.

Mason[4] agrees that the period and magnitude of these orbital changes lead to solar energy fluctuations which can explain parameters of the palaeoclimatological record, such as global ice volume change.

(3) Greenhouse effect—modification by CO_2: Global increases in the amount of CO_2 in the atmosphere through the burning of fossil fuels are thought to be important enough to influence the greenhouse effect (the process by which infrared radiation is trapped by CO_2 and water vapour leading to surface temperatures higher than the black body temperature). Although the direct effect of an increase in CO_2 may be quite small (surface temperature rises of about 2 K if the amount of CO_2 is doubled), it is difficult to estimate how the rest of the system will react. For instance, a small global temperature increase with the addition of CO_2 will increase the moisture holding capacity of the air. Water vapour is the most important constituent involved in the greenhouse effect and so the net increase in temperature could be much greater than that predicted on the basis of CO_2 alone.

(4) Volcanic dust: The presence of volcanic dust in the upper atmosphere has been put forward as a way of increasing the albedo and thus reducing the mean atmospheric temperatures. Hunt[5] has investigated such influences with a three-dimensional general circulation model and has found a mean surface temperature reduction of at most 1 K. He concludes that volcanic eruptions could have a significant effect on the atmosphere, though lasting changes are improbable.

(5) Ice cap/albedo positive feedback: Climatology literature abounds with possible positive feedback mechanisms which could render the climate unstable. Of these, the polar ice cap/albedo feedback is the most popular. The argument is quite simple. An increase in the horizontal extent of the polar ice sheet increases the global albedo, which in turn results in lower temperatures through the reduction in absorbed solar radiation. Colder surface conditions encourage the expansion of the ice sheet and so on. Budyko[6] and Sellers[7] have investigated the equilibrium mathematical solutions of simple zonally averaged heat balance models which have ice sheet dependent albedo variation and include a diffusive poleward transfer of heat. Their calculations show that a decrease in the solar irradiance of 2–5% would be sufficient to initiate another ice age.

(6) Oceanic influences: Weyl[8] has presented a case for the initiation of Ice Ages by reduction of the salinity of the North Atlantic Ocean. He claims that the 'Little Ice Age' of AD 1500–1850 substantiates his hypothesis. Certainly on a time scale of years or tens of years the ocean surface temperatures are believed to exert a strong influence on weather types. Namias[9] believes that the evolution of monthly mean weather is largely controlled by the oceanic temperature anomalies.

(7) Other topographical influences: Changes in the condition of the Earth's land surface by mountain building and vegetation growth or decay are amongst other possible climate change mechanisms. Charney[10] has described a positive feedback mechanism which causes desert conditions through overgrazing: reduction of plant cover increases the albedo which, in turn, causes a greater radiative deficit. The resulting horizontal temperature gradients induce subsidence which inhibits convective precipitation and therefore curbs plant growth.

Methods of climate modelling

It is possible to divide climate models into three broad categories: (1) energy balance models that include radiation/albedo feedback processes but contain no explicit dynamics; (2) low resolution statistical–dynamical models that include large scale eddy transports of heat and momentum in parametric form, for example, as some curious variety of conductivity; and (3) simulation models of very high resolution that include all motion on scales greater than some 100–200 km.

(1) The energy balance models can be used to explore the sensitivity of zonally averaged climatic states that are determined by the ice-cover/albedo feedback mechanism (see Fig. 1).

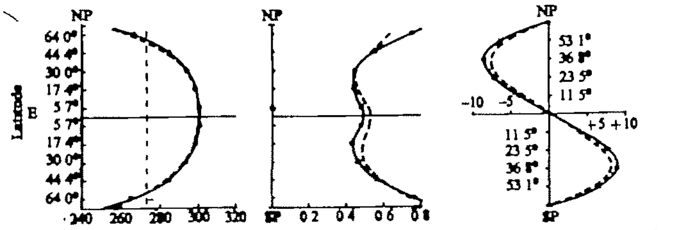


Fig. 2 Taken from Paltridge[12]. ——, Predicted; ----, observed

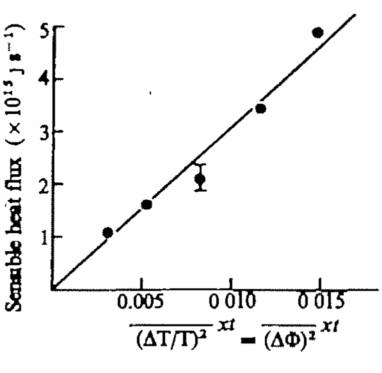
They show that small changes in the solar input can cause catastrophic changes in the surface temperature of the model. Using a time-dependent model, Schneider and Gal-Chen[11] have found that a reduction in the intensity of solar radiation by more than 1.6% results in a fall of global mean temperature of over 100 K. As with all climate models, it is difficult to believe that such large excursions from present conditions are adequately represented by the processes, the parameterisation of which depends on the current state of the atmosphere.

A unique approach was adopted by Paltridge[12] who sought a minimal principle governing the zonally averaged distribution of cloud cover, surface temperature and poleward heat transport. The incident solar radiation at the top of the atmosphere was the only externally prescribed quantity. By minimising the rate of generation of entropy, very close agreement of the fundamental model unknowns with observation was found (Fig. 2). No dynamical information was required in the model—a disturbing fact when so much effort is devoted to integrating the equations of fluid motion. Further work by Paltridge[13] has led to predictions of the global cloud cover and rainfall, again with surprising accuracy.

The notion that the climate does not depend on the Earth's rotation seems hard to accept, yet the agreement with observation is striking. The philosophy of such an approach is very appealing, and is in line with the idea of applying thermodynamic concepts to the climatic problem. Indeed, the principle of minimum rate of generation of entropy is well established in the field of non-equilibrium thermodynamics for a certain class of physical processes[14].

(2) These models are primarily concerned with the main global features of the time-averaged wind and temperature fields without explicitly describing the transient depressions and anticyclones or any other smaller scale of motion. The statistical effect of these smaller scales of motion is parameterised in terms of the larger scale fields which are described explicitly. One such 'prescription' for the turbulent transfers of heat and momentum accomplished by large scale eddies was given by Green[15]. Some aspects of this parameterisation seem to describe global-scale transports realistically (Fig. 3). The parametric formulation of the eddy transport of angular momentum is not so realistic and remains one of the most important and difficult aspects of our understanding which is highlighted in these models.

Fig. 3 Eddy flux of sensible heat across 50°N plotted against $\overline{(\Delta T/T)}^{2*}$, $(\Delta T/T)$ = fractional temperature difference between 20 and 70°N, 500 mb (from Green[15])



(3) The general circulation models of this category are widely used for climatic studies. They are essentially similar to the models used for weather prediction and seek to embrace as many relevant physical processes with as much resolution as is computationally feasible. In contrast to the previous type, these models extend their explicit representation to that of weather patterns, even though in their weather forecasting capacity they have predictive value for only a few days. In the most advanced models, the circulation of the oceans is also modelled, though integrated with a much longer time step to that of the atmospheric circulation.

Uses and abuses of models

The Budyko–Sellers models are useful for assessment of sensitivity: most of the estimates of the effect that changes in CO_2 or solar irradiance might have are based on them. Unfortunately, there is only the insufficient climate record against which they might be tested. This is true of most models and has the corollary: "any fool can make climate predictions because he won't be there when it's time to collect the bad debts."

The Paltridge model apparently gives excellent results, but no-one understands the physics that goes into its construction. Furthermore, it has only been tested against the present climate of the Earth, and might, therefore, be just a one-off success. Highly parameterised models can reproduce some results of more complex models (though not as well as that of Paltridge) but so far are generally considered to be an outside chance in view of their palpable over-simplification. General circulation models (GCMs) have been extensively used (The UK Meteorological Office spends about as much on running their model as goes into university education of meteorologists in Britain.) Much is known about their behaviour which is not known about other models. The nature of these characteristics may form a guide to those of other models.

All models are much more similar to each other than they are to the real atmosphere—it's easier to forecast the weather forecast than the weather. Details are consistently wrong in all models.

Although the predicted values for the poleward transport of energy and momentum are surprisingly accurate (they are closer to the long-term mean than the year-to-year variability of atmospheric transfers), the calculated intensity of the eddy motion is much less than observed.

Earlier versions demanded some judicious manipulation of parameters in order to reproduce the present climate. That this is said not to be true of later models may indicate greater skill in anticipating the required numbers or greater understanding of the relevant physical processes. How can we tell?

All GCMs produce westerly jet streams that extend much too far into the upper atmosphere. A detail, but it goes with not having the polar stratosphere warm enough by some considerable amount. It is worrying that this type of defect is tangible and universal in a region where we 'know all the physics'.

No climatic model has actually predicted a climatic anomaly or has been able to simulate one for which there was sufficient meteorological data to make it a proposition in numerical analysis. We believe that this applies, for example, to the anomalous North American winter of 1977–78, and to the British drought of 1975–76.

Finally, these models suggest that climate is essentially unpredictable: that we can only predict in the probability sense of many 'realisations' (or re-runs from 'nearly-the-same' initial data). Is it the climate model or climate that is unpredictable? We must stress that these conclusions are biased and arguable. Some are demonstrably true of general circulation models, but we believe many are symptoms of the climate problem.

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Numerical simulation of climate and climatic change

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Detailed three-dimensional numerical models of the atmosphere, coupled as necessary to models of other parts of the climatic system, provide the most promising approach to understanding the physical basis of climate. Models of this kind can be used to investigate the impact of anthropogenic pollution on climate. At the present time, the main concern is with increasing concentrations of CO_2 which might lead to overall warming of the troposphere, but chemical and thermal pollution may also pose a threat. The possible climatic changes would take place slowly and would involve the response of the slowly reacting parts of the climatic system, particularly the oceans. The problem of how to simulate such changes of climate presents many difficulties, which are currently being studied.

THE most powerful tool available for studying the physical basis of climate and climatic change is the general circulation model, which simulates the evolution of the atmosphere numerically and is capable in principle of taking into account all the factors that determine climate. Such models are complex and require a range of expertise and prolonged experimentation to bring them to the point where they can be put to good use in climate experiments that are of value. Consequently, their development has been undertaken at only a few centres. At a recent conference in Washington (April, 1978) organised by the Joint Organising Committee for the Global Atmospheric Research Programme for the purpose of comparing climatic simulations, results were presented from nine institutions. Of these, four were from the USA, two from the Soviet Union, with one from Australia, Canada and the UK. However, some of the models described were at a preliminary stage of development and only the American and UK Meteorological Office groups have tested models extensively in studies of climate.

Fundamental physical equations

The starting point for general circulation models are the fundamental physical equations for the rates of change of the components of velocity, temperature and humidity at a point in the atmosphere. The equations include a description of the processes by which the atmosphere interacts with radiation (infrared and solar) and the underlying surface of the Earth. They are solved on a network of points over the globe to provide a continuing evolution of atmospheric states. Processes that take place on too small a scale in the real atmosphere to be represented explicitly are 'parameterised' in terms of the variables available on the mesh; for example, the vertical transfer of heat and moisture due to buoyancy forces which in the real atmosphere produce cumulus clouds and showers is treated in this way.

Conditions that are external to the atmospheric dynamical system are specified. These include the constituents of the atmosphere, the radiation conditions, defining either explicitly or implicitly the Sun's position with respect to the Earth, and the characteristics of the Earth's surface that influence its interaction with the atmosphere and radiation. For a land point, the latter would include the height above mean sea level, the albedo without snow cover and the change due to snowfall, the roughness, and a heat capacity to define how its temperature would respond to the absorption of heat.

For a sea point a purely atmospheric model lacking a dynamical interaction with the ocean requires either the sea-surface temperatures to be specified or to be determinable from the local heat balance. The former, with the values set to the long-term climatological average, is appropriate when the object is to provide a realistic simulation of climate, and the latter has been used in attempts to understand particular features of the atmosphere's general circulation where the use of temperatures that are themselves partly determined by the atmospheric circulation is inappropriate. Combined ocean–atmosphere models which allow a dynamical evolution in both fluids are now being constructed and tested[1], as for many processes of interest in climate an interactive system is essential.

General circulation models

These provide in principle, and already to some extent in practice, a method of estimating the global climate for any set of prescribed 'external' conditions. To do so, they have to be integrated through an extended period of simulated time during which many atmospheric states are created. The length of integration needed may well depend on the precise problem being considered. It should be long enough for a statistical equilibrium to be established between the atmosphere and the prescribed conditions and, ideally, it should include a sufficiently varied range of atmospheric states to make it possible to estimate the probability of occurrence of any particular state with these conditions.

Given this information, the more usual characteristics that are taken to define climate may then be derived; these are the long-term averages, the extremes, the year-to-year variability, and so on. For most problems involving a change of climate, it would probably be more convenient to estimate the climate, not in absolute terms, but with reference to what is now observed. This is an easier goal, and as minor deficiencies revealed by model estimates of the present climate can probably be allowed for, the end-results may be achieved with rather simpler models.

Models are still in a comparatively early stage of development and up to the present attention has for the most part been concentrated on their ability to simulate the average conditions. For example, a number of simulations of the present climate have been described in the literature[1,3–6], and there have been attempts to simulate the climate of the last ice-age by using data from the CLIMAP project[7] to define the extent of glaciation and the sea-surface temperatures 18,000 yr ago. In general terms the

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models achieve a simulation of present climate that contains all the major features of the atmospheric circulation, but there are systematic errors in the intensity of some of them that call for further development and improvement.

The importance of this aim has been recognised by the international community which has supported international experiments such as GATE (the GARP Atlantic Tropical Experiment) in 1974 and FGGE (the First GARP Global Experiment) in 1978–79, the main purpose of which has been to obtain the data required to test and systematically improve the simulations. An important aspect of the FGGE is that data will be collected from a complete yearly cycle, enabling the models to be tested in a wide variety of conditions. Remembering that within the annual cycle there is a greater range of climates than have affected any particular part of the year at least in the last millenium, it is obvious that the potential of the FGGE to improve climatic modelling, provided a sufficiently comprehensive coverage of observations is obtained, is substantial.

The ice-age simulations[8–10] were obtained using similar methods to those applied to present-day climatology. Maintaining the sea-surface temperatures, the distribution of ice and the solar parameters at their 18,000 yr BP values, the general circulation models were integrated long enough for the atmospheric motions to come into statistical equilibrium with the boundary conditions. There are significant differences in the simulations, and because of a number of inadequacies the results must be considered indicative rather than definitive. Nevertheless, they are of interest in suggesting certain aspects of the ice-age climate that can be checked against geological evidence. Thus, the GFDL integration[8] favours comparative dryness in the ice-age tropics and this is considered to be supported by evidence provided by the sand-dune distribution of 18,000 yr BP. The model integrations agree in having generally less rain and lower global temperatures during the ice-age than now.

Why numerical models?

It is evident that although general circulation models are a very powerful tool for investigating the physical basis of climate and climatic change, they involve a lot of computing, and therefore it is tempting to enquire whether there might not be a simpler method which could be equally successful. There are, however, good reasons why large numerical models occupy a central position in studies of climate. The first stems from the fact that controlled experiments cannot be carried out on the atmosphere, at least not on anything approaching a global scale. In these circumstances, large numerical models provide the only feasible method of testing hypotheses. Without them research in climate can easily become (and indeed for a long time was) a series of uncheckable speculations. This is not to imply that the results of a particular model are correct; but it is an inherent property that they provide not simply an initial cause and a subsequent effect, but a chain of consequences linking the two. Taken individually, the links in the chain are usually of such a kind that they can be checked quantitatively against atmospheric observations; in particular, critical links can be identified. Another reason is that it has become clear that climate is determined by complex interactions among a number of systems, these being the atmosphere, the oceans, glaciers, the Earth's surface, and so on. Indeed, the essence of the problem of climate which distinguishes it from, for example, problems in atmospheric dynamics, is that interactions between the atmosphere and other systems are involved. The processes occurring within the systems are themselves often exceedingly complex; to rationalise and explain the interactions between them is generally speaking beyond the expertise of any one individual and can involve so many factors whose importance cannot be ruled out *ab initio* that the only possible approach is to try to incorporate them in a model that can be solved numerically.

These points can be illustrated from the work of Charney[11] in trying to explain the severe drought in the Sahel in 1972. The range of possible interactions that might have created the drought is very large, and they cannot be subjected to precise experiments; it may therefore be very misleading to choose certain of them as dominant without quantitative modelling of all the potentially important factors. In the first instance Charney[11] sought to explain the Sahel drought by a biogeophysical feedback mechanism. He postulated that a reduction of vegetation in a borderline desert region had occurred, possibly due to lack of rain or to heavy cropping by animals. A greater amount of light soil would therefore be exposed and the increase in the albedo would lead to a greater proportion of the incoming solar radiation being reflected back to space. Less heat would therefore be available to heat the atmosphere. If temperatures further from the desert remained unchanged, a more intense circulation with downward motion over the area of increased albedo would tend to be produced, and so reduce the rainfall and the vegetation cover even further. Charney proposed that this could account for the prolonged drought in the Sahel.

The hypothesis was later tested in experiments with a general circulation model[12]. In this case the situation was shown to be much more complex than the original explanation allowed for. Thus, it was found that, as expected, a larger albedo reduced the proportion of the incoming solar radiation absorbed at the surface, but because it also reduced the input of heat and moisture to the lower atmosphere there was less cloud. More solar radiation therefore, reached the ground, and in some experiments the absorption of solar radiation at the Earth's surface actually increased. However, with reduced cloudiness there was less infrared radiation back to the surface, with the result that the net absorption of radiation was less. The proportions that went into evaporation and into sensible heating of the atmosphere depended on the wetness of the surface; in the experiments this factor was fixed from climatological values, but in reality it depends on the rainfall, thus introducing another feedback mechanism that is potentially very important. In general, increasing the albedo led to a decrease in the precipitation, but this was not invariably the case. In experiments in which the evaporation was almost negligible and which therefore accorded most nearly with Charney's original conjectures, the results varied among the regions where the albedo was increased; the change seemed to increase precipitation in some areas and reduce it in others.

It is clear from Charney's experiments that the consequences of an increase in albedo are by no means easy to predict. An assessment of the physical processes involved requires rather precise quantitative modelling and a careful check against observations of all the interactions that seem to be important. From the point of view of explaining the Sahel drought the situation is even more complex, as there are factors other than the albedo that may be important. Thus, Walker and Rowntree[13] have shown that the soil moisture content can have a direct influence on atmospheric dynamical developments in desert fringe areas, and this factor was not represented effectively in Charney's experiments. Also, from studies of the effects of anomalous sea-surface temperatures on the atmosphere, carried out using the Meteorological Office general circulation model, there is some evidence that the droughts may be linked with abnormally cold water in the tropical Atlantic. If this is confirmed it might account for the longevity of some droughts in the Sahel, as there is considerable evidence of long period variations in tropical sea temperatures.

The processes that determine climate evidently involve delicate balances among physical systems which even in isolation are complex and not easily understood, and which cut across the boundaries between the individual expertise of scientists. The problems can only be solved as a result of effective cooperation between meteorologists, oceanographers, radiation experts, plant physiologists, and so on. General circulation models provide a means of integrating the contributions of each field of expertise to arrive at the total influence on climate.

Impact of man's activities

In considering climatic change due to natural causes, the existence of climatic data for the past, either as direct measurements or as proxy data derived from tree-rings, ice-cores or cores from ocean or lake sediments, provides at least a statistical account of the variations that are likely. When one turns to the possible impact of man's activities, there is little chance of finding a sufficiently close historical analogy, and it is difficult to envisage a systematic approach to estimating the effects quantitatively which does not depend essentially on simulating them by means of sufficiently comprehensive numerical models. At the present time, for example, the main concern arises from the increase in CO_2 concentrations in the atmosphere, thought to be due principally to the burning of fossil fuels. One-dimensional column models, which calculate the local temperature change in a still atmosphere due to varying concentrations of the radiatively active gases, indicate that, for typical conditions, increasing the concentration of CO_2 produces a warming of the troposphere and a cooling of the stratosphere and upper atmosphere.

There is now substantial agreement[14] among such models that the temperature change to be expected from a doubling of the amount of CO_2 from its present value of about 320 p.p.m. is about 2 K at the Earth's surface. This value includes a feedback from water vapour which accounts for about half the total increase. The CO_2 warms the lower atmosphere, which in consequence can hold more water vapour, and the absorption of radiation by the water vapour enhances the warming. The results depend on the conditions of cloudiness assumed, and in general no allowance is made for changes in cloudiness that might arise from the increased temperature and water vapour content.

A general circulation integration by Manabe and Wetherald[15] using a much simplified surface topography and ignoring the effects of ocean currents, the annual solar cycle and variations in cloudiness, has demonstrated additionally that in higher latitudes, where the warming reduces or removes highly reflecting snow-cover with its typical low level atmospheric inversion, the change in surface temperature could be much greater, perhaps up to about 10 K. They also found that the hydrological cycle was more intense because of the additional moisture-carrying capacity of the atmosphere, and it can be surmised from their paper that the distribution of rainfall was significantly altered. A statement approved by the Executive Committee of the World Meteorological Organisation[16] has called particular attention to the CO_2 problem, and has pointed out the urgency of defining its impact more precisely.

The immediate problems are, firstly, to forecast future concentrations of CO_2 and, secondly, to translate their effects into likely changes in climate. It is on the second problem that meteorological investigations must concentrate, although it has to be recognised that changes in climate may affect the storage of CO_2 in the oceans and in the biomass; therefore, the two aspects are not independent. The obvious first extension from present results is to try to obtain a clearer view of the regional changes, particularly of temperature and rainfall, that might be produced by increased amounts of CO_2. It is perhaps salutary to recall the results of a Meteorological Office experiment in which the Arctic was maintained ice-free[17]. This of course led in general to higher temperatures. Nevertheless, Britain and Western Europe were much colder because the predicted atmospheric flow patterns showed prevailing winds that were backed from their present direction towards a more easterly point. In a similar way, the effect of more CO_2 will probably vary from one part of the globe to another, some parts showing a cooling in spite of the global temperature being higher. There might also be significant changes in the probabilities of extreme events, such as the incidence of very cold winters or severe droughts in Britain, or of flooding of low-lying areas. It is important to try to identify these regional variations, with perhaps special emphasis on the rainfall changes which could easily have a substantial impact on agricultural productivity.

If present indications are correct, the effect of greater CO_2 concentrations will be felt increasingly over the next century or so. There will be a gradual change in the Earth's climate involving changes in the oceans, in sea-ice, in glaciers and in the vegetative cover of land surfaces as well as in the atmospheric motions. Just how radical the changes will be is uncertain at this stage. To produce a forecast of the evolution of the climate with time is a problem which may not be capable of solution in an exact sense, and for which indeed no clear methodology that would lead to a solution has yet been established. To create a model involving all the systems analogous to an atmospheric general circulation model and simulate their interactions comprehensively in a long-term integration, for example, is out of the question.

Estimates of future climate

There are, nevertheless, approaches that might be tried. It has already been emphasised that general circulation models provide a means of estimating the climate for a given set of 'external' conditions. We have to reckon with the fact, however, that few parts of the climatic system are truly external and independent of the atmosphere. Factors emanating from outside the Earth, such as solar radiation, factors emanating from below the Earth's surface, such as volcanic dust, and certain inputs by man himself, come into this category. There remains a complex of systems that interact with the atmosphere; some react quickly, whereas others change only slowly as a result of an atmospheric input.

The atmosphere itself is highly variable from day to day, and some aspects of the processes at the Earth's surface such as evaporation and the establishment or disappearance of temporary snow-cover are on a similar time scale. At the other end of the scale, glaciers and the deep ocean may require years of altered conditions to change significantly. From the point of view of establishing an estimate of climate that is essentially steady (apart from the annual cycle) over periods of years or decades, the very slowly responding systems can probably be considered as effectively external. A reasonable methodology for dealing with slow long sustained climatic change then emerges.

The first step would be to establish the statistical characteristics of a climate for a relevant set of 'external' conditions using a general circulation model that includes factors varying on short time scales. As well as the atmosphere and some land surface variations, this ought to include also the upper layers of the ocean, which react quickly to atmospheric changes. The existence of upwelling in the oceans by which in limited localities the deep waters can come fairly rapidly into contact with the atmosphere, might cause difficulties which would have to be overcome. The climate once established could then be allowed to act on the more slowly varying parts of the climatic system for as long a period as was deemed reasonable. For example, the gradual change in the ocean circulation and changes in glaciation could be calculated on the basis of a steady climate as long as the changes were not too large.

The assumption is essentially that for small changes the variations in the slowly responding systems are nearly linear. The extent to which this is so could of course be checked by preliminary experiments. Once significant changes had taken place it would be necessary to go back to the general circulation model to re-establish a relevant climatology. In this way it is at least methodologically possible to try to estimate the evolution of climate taking place over long periods of time.

The CO_2 problem is the most pressing at present, because there is enough generally accepted scientific evidence to indicate that the impact on climate could be substantial. Changes that might result from other human activities are less clearly understood, but there are obvious possibilities that they might become more important in the future. Thus, the input of waste heat into the atmosphere has significant effects locally, evident for example in the increase in temperature at some meteorological sites as a result of urbanisation nearby.

The future global energy position is far from clear, but if this involved the use of very large nuclear power stations, vast amounts of heat could be injected into the atmosphere in concentrated sources whose effects are as yet not well understood. An experiment using the Meteorological Office general circulation model, carried out in association with the International Insitute for Applied Systems Analysis, has shown that the input of 1.5×10^{14} W to a particular area had a large effect on the global climate and changed the pattern of air movement and rainfall very substantially. This amount is much greater than present world consumption (~ 7–8×10^{12} W); nevertheless, the response is such as to demonstrate that it would be prudent to investigate the possible impact on climate before large energy projects which might release large amounts of energy are embarked on.

The possibility that the ozone layer might be reduced by the injection of pollutants into the stratosphere has been much discussed in recent years, first in the context of supersonic aircraft flying in the stratosphere and more recently as a result of the accumulation of inert gases such as freons. The possible danger is firstly to living material because of the increased amounts of ultraviolet radiation reaching the ground, and secondly, in the longer term, to possible changes in climate that this would produce.

Some of the early fears about increased radiation were evidently exaggerated[19], and the mechanism by which changes in ozone would lead to noticeable changes in the climate is far from clear. Some experiments[20] in which the ozone was increased showed substantial warming at the ozone levels themselves but little impact at the Earth's surface. Should the possibilities for climatic change be shown to be more real than has so far been demonstrated it would be necessary to follow a methodology similar to that just outlined for CO_2 to make some estimate of the detailed effects over a long period.

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Recent developments in atmospheric chemistry

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The chemistry of the atmosphere was a largely neglected topic until the last decade during which concern about pollution has produced a flurry of activity. The processes which may deplete the relatively small amount of ozone in the stratosphere which protects us from harmful UV light have much in common with those in the Los Angeles-type smog which generate ozone and other oxidants. Such systems involve chain reactions in which free radicals such as HO, HO_2, ClO can carry out a chemical conversion process (ozone to oxygen) many times before they are destroyed. Recent research shows that nitrogen oxides from the exhausts of supersonic aircraft or derived from increased use of nitrogenous fertilisers will have little effect on stratospheric ozone but the potential effects of chlorine compounds, such as aerosol propellants, require careful examination. In contrast, the oxidation of carbon monoxide is now thought to be a major source of ozone in the lower atmosphere.

THE past decade has seen a tremendous growth in research on atmospheric chemistry; this has stemmed largely from the need to identify the processes which remove many of the compounds which man releases into the atmosphere on a large scale and to assess their potential effect on the environment. These problems highlighted our lack of knowledge of the concentrations of important trace species in the unperturbed atmosphere and of the processes which control them. Fortunately both atmospheric and laboratory measurements on these trace species have been greatly aided by recent developments in highly sensitive spectroscopic methods for measuring their concentrations, notably fluorescence, high resolution absorption spectroscopy and laser magnetic resonance.

Computer modelling provides an essential link between laboratory measurements of reaction rates of atmospheric species and the determination of their concentrations in the atmosphere, as the number of reactions is large and the atmospheric circulation a formidably complex problem. Because, in the lower and middle atmosphere, gases are transported by the movement of air masses rather than by molecular diffusion, it will be several years before the massive computational problem of combining a general circulation model of the whole atmosphere with an adequate description of the chemistry can be achieved. In most current work, the transport is parameterised in terms of an 'eddy transport coefficient' which has the same dimensions as a diffusion coefficient.

Two-dimensional models of the atmosphere in which altitude and latitude are variables are now replacing the one-dimensional models involving only altitude; these cannot, for instance, describe the transport of ozone from its predominant formation regime near the equator to the poles where its concentration is greatest.

Comparisons of the measured and calculated concentrations of trace species in the atmosphere provide the best test of the adequacy of our understanding of atmospheric chemistry and transport. Because the tropopause (the temperature minimum at an altitude of about 15 km) provides an effective barrier to transport between the dry, relatively stable stratosphere and the moist rapidly mixed troposphere below it, it is convenient to consider the chemistry of these regions separately (Fig. 1).

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Stratospheric chemistry

Here the dominant interest is in the processes which control the small amount of ozone which protects the Earth's surface from ultraviolet light with wavelengths less than 300 nm; this corresponds to a 3-mm thick layer of pure ozone gas at ground level. The basic mechanism for the formation and removal of ozone was proposed by Chapman[1] in 1930. Molecular oxygen is dissociated by light with wavelengths less than 242 nm to form oxygen atoms. These combine rapidly with molecular oxygen to form ozone, a process which requires a third body (M) to remove excess energy

$$O_2 + h\nu(\lambda < 242\ \text{nm}) = O + O \qquad (1)$$

$$O + O_2 + M = O_3 + M \qquad (2)$$

Ozone is very rapidly photolysed by visible and ultraviolet light to regenerate oxygen atoms

$$O_3 + h\nu = O_2 + O \qquad (3)$$

It is also removed by reaction with atomic oxygen to regenerate molecular oxygen

$$O + O_3 = O_2 + O_2 \qquad (4)$$

There is a fundamental difference between these two decomposition processes for ozone. Process (3) followed by reaction (2) merely interconvert O and O_3 which are termed 'odd oxygen' species, whereas (4) converts the odd oxygen formed by process (1) back to (even) molecular oxygen. Although Chapman himself and others had clearly envisaged that other processes could destroy much atmospheric ozone, only in the present decade have laboratory and atmospheric measurements become accurate enough to establish that reaction (4) only accounts for about one-fifth of the odd oxygen destruction in the atmosphere[2].

The other mechanisms for ozone removal can most easily be envisaged in terms of catalytic cycles in which a species converts O and O_3 into O_2 without being destroyed itself

$$X + O_3 = XO + O_2 \qquad (5)$$

$$XO + O = X + O_2 \qquad (6)$$

$$O + O_3 = O_2 + O_2 \qquad (5)+(6)$$

For reactions (5) and (6) to be rapid, X needs to have an unpaired electron; the importance of atmospheric cycles with X = H, HO, NO and Cl have been recognised[3–6]. (The stability of HF in the atmosphere prevents F contributing to such cycles; there is insufficient bromine in the atmosphere for X = Br to be important.) The hydrogen radicals, H, HO and HO_2, which participate in the X = H and X = HO cycles come mainly from the attack on water vapour of excited oxygen atoms, $O(^1D)$, formed by the photolysis of ozone at wavelengths below 310 nm. These radicals are removed by mutual combination reactions such as

$$HO + HO_2 = H_2O + O_2 \qquad (7)$$

$$HO_2 + HO_2 = H_2O_2 + O_2 \qquad (8)$$

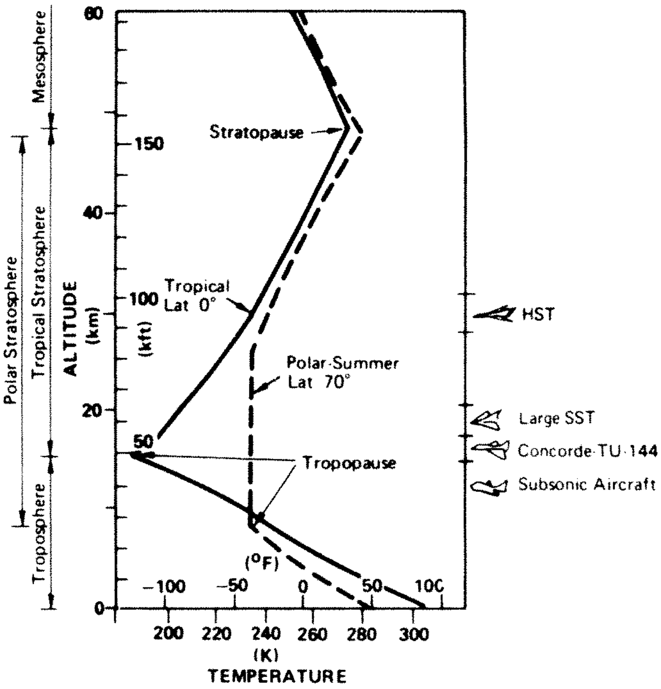


Fig. 1 Temperature of the atmosphere as a function of altitude.

Figures 2 and 3 illustrate the basic corresponding cycles for X = NO and X = Cl (ref. 7). Here man-made compounds may contribute to ozone destruction. Increased use of nitrogenous fertilisers could lead to significantly increased levels of nitrous oxide in the atmosphere, but the natural sources and sinks of nitrous oxide (N_2O) in the troposphere are not well enough known for an assessment of this effect. The amounts of NO and NO_2 in the stratosphere (totalling about 1 part in 10^8) are also enhanced by their presence in the exhausts of high flying aircraft, but 'rain out' in the troposphere prevents the vastly greater quantities of NO and NO_2 released by combustion processes at or near ground level from reaching the stratosphere. Even allowing for the fact that the efficient decomposition of NO_2 by near ultraviolet sunlight and the subsequent reaction (2)

$$NO_2 + h\nu(\lambda < 400\ \text{nm}) = NO + O \qquad (9)$$

$$O + O_2 + M = O_3 + M \qquad (2)$$

$$NO_2 + O_2 + h\nu = NO + O_3 \qquad (9)+(2)$$

effectively reverse the ozone destruction process

$$NO + O_3 = NO_2 + O_2 \qquad (10)$$

the nitrogen oxides provide the strongest removal process for odd oxygen. However, man's efforts in adding to the stratospheric burden of nitrogen oxides or chlorine compounds cannot be assessed directly from this, as recent laboratory studies[8,9] have established the rapidity of the reaction

$$HO_2 + NO = HO + NO_2 \qquad (11)$$

which, like the closely related process

$$ClO + NO = Cl + NO_2 \qquad (12)$$

does not itself remove odd oxygen, but links the odd oxygen destruction cycles so that the effects of nitrogen oxides, halogens, and so on, on ozone are not additive. On this basis it is believed that current and forecast amounts of stratospheric flight (for example, by Concorde and Boeing SP747) will have a negligible effect on stratospheric ozone, but the effects of chlorine compounds are magnified[9–11].

The present levels of chlorine in the stratosphere (1.5–2 parts in 10^9) arise mainly from methyl chloride[12] (from algae and from slash-and-burn agriculture), from salt spray and from volcanic gases. These sources will soon be overtaken by the chlorofluoromethanes $CFCl_3$ and CF_2Cl_2 now present at 1 and 2 parts in 10^{10} respectively in the troposphere[13]. These compounds, which are used as aerosol propellants, refrigerants and foam-blowing agents, have no known natural removal process other than transport into the stratosphere followed by decomposition to yield chlorine atoms, and eventual removal as hydrogen chloride, a process which takes 5–10 years. However, 90% of the halocarbon will remain in the troposphere under uniform mixing conditions because this region contains 90% of the molecules in the atmosphere. The atmospheric lifetimes of $CFCl_3$ and CF_2Cl_2 are therefore believed to be 50–100 years, over which period the depletion of ozone is predicted to increase to near 15% if these substances continue to be released at current rates. However, uncertainties in the rate coefficients of the 50–100 reactions involved and in the transport parameters each contribute a factor of two to the uncertainty of such estimates[7,10]. Another source of uncertainty is the 'unknown chemistry' involving perhaps compounds such as HO_2NO_2, $ClONO_2$ and HOCl which have very limited stability in the laboratory but might be present in the stratosphere in amounts approaching the current detection limits (about 1 part in 10^9).

Verification of the model calculations depends on reliable measurements of trace species in the atmosphere which, with the exception of ozone, are few in number and show significant fluctuations apparently associated with the past history of the air masses sampled. For this reason, simultaneous measurements of a number of trace species are now considered particularly important, as they can provide a better test of the correct identification of the reactions interconverting these species. For instance, the ratio of NO_2 to NO at 20–25 km altitude is predominantly controlled by the rates of reactions (9), (10) and (13)

$$O+NO_2=NO+O_2 \qquad (13)$$

giving

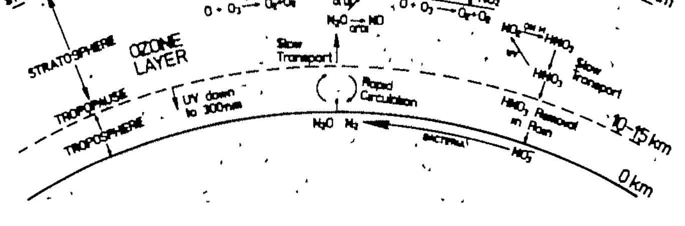
$$\frac{[NO_2]}{[NO]}=\frac{k_{10}[O_3]}{J_9+k_{13}[O]}$$

the ratio of $[O_3]$ to [O] being governed by processes (2) and (3). Thus, the measurement of the ratio $[NO_2]/[NO]$ at these and at higher altitudes where reactions (11) and (12) become significant can verify the accuracy of our understanding of stratospheric chemistry. For these species, the measurements are normally carried out by long path infrared spectroscopy of the setting sun when the ratio of NO_2 to NO is increasing rapidly because the photolysis of O_3 and NO_2 is declining markedly and agreement with the model is not yet perfect[14]. Anderson *et al.*[15] has measured Cl and ClO concentrations at altitudes of 25–40 km by balloon flying and parachuting a flow apparatus in which chlorine atoms are detected in fluorescence either as initially present or produced by reaction (12). The values which he finds are too high to be readily reconciled with measurements of HCl at lower altitudes (with which it should be in steady state, as indicated in Fig. 3) or with known ozone concentrations. This and evidence for a seasonal variation make simultaneous measurements of Cl, ClO, HO and O or O_3 a high priority experiment.

Tropospheric chemistry

Here, as with stratospheric chemistry, much of the recent research has been governed by pollution problems. Historically, sulphur dioxide has been the most important air pollutant in the UK and although its concentration in urban air has been greatly reduced by the use of alternative fuels and by dispersal through high chimneys, the mechanism of its oxidation over a period of weeks before its deposition as sulphuric acid in rain or as sulphate aerosol is far from understood. Homogeneous reactions involving combination reactions with HO in unpolluted and HO_2 in polluted atmospheres are believed to be important and there is evidence for a catalytic process involving transition metals, notably manganese, in polluted atmospheres[16].

Fig. 2 Dominant ozone formation and removal processes in the natural atmosphere.



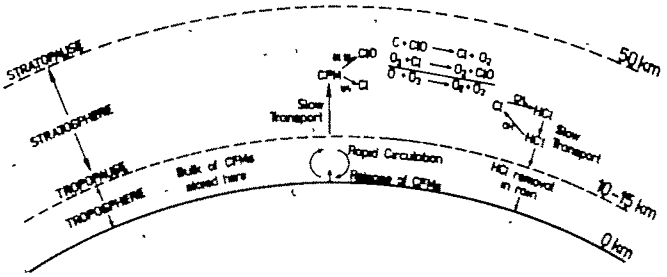


Fig. 3 Simplified diagram of the behaviour of chlorofluoromethanes in the atmosphere.

With an increase in the use of cars, the place of the traditional London fog is beginning to be taken by photochemical smogs which have been a familiar problem in the USA for at least 20 years. These occur when a temperature inversion in sunny weather prevents the dispersal of the exhaust gases of internal combustion engines. The photolysis of NO_2 (reaction (9) above) produces oxygen atoms, these and the ozone which they produce by combination with molecular oxygen (reaction (2)) attack particularly the unsaturated hydrocarbons which are also present in car exhausts. This initiates a highly complex series of oxidation reactions involving particularly the free radicals HO and HO_2 which yield *inter alia* aldehydes and the hitherto unknown peroxy-acyl nitrates ($RCO.OONO_2$), which like the ozone also present are powerful irritants. Carbon monoxide is also oxidised to carbon dioxide. Computer models of the very complicated series of reactions which are believed to occur in smog can now reproduce the smog in laboratory chambers from known materials and also the diurnal variation of the main species involved in the atmosphere, but many individual steps require laboratory measurements[17]. A recent development has been the detection of nitrosamines in Los Angeles smog when sources of aliphatic amines are present[18].

Anthropogenic sources of ozone may well be important even in apparently unpolluted regions of the lower atmosphere. Fishman and Crutzen[19] have recently pointed out that the preponderance of land in the Northern Hemisphere results in three times as much ozone destruction at the surface there compared with the Southern Hemisphere but there is no evidence of a corresponding ratio of ozone fluxes downwards from the stratosphere; this would be required if this were the main ozone source. They conclude that there must be significant tropospheric sources of ozone in the Northern Hemisphere and suggest that these are anthropogenic: the oxidation of hydrocarbons and the oxidation of carbon monoxide

$$CO+HO=CO_2+H \qquad (14)$$

$$H+O_2+M=HO_2+M \qquad (15)$$

$$HO_2+NO=HO+NO_2 \qquad (11)$$

$$NO_2+h\nu=NO+O \qquad (9)$$

$$O+O_2+M=O_3+M \qquad (2)$$

giving

$$CO+2O_2=CO_2+O_3$$

As in the stratosphere, the HO radicals which catalyse these oxidations are produced by the reaction with water vapour of a small portion of the excited oxygen formed in the photolysis of ozone at short wavelengths

$$O_3+h\nu(\lambda<310\ \text{nm})=O_2+O(^1D) \qquad (16)$$

$$O(^1D)+H_2O=HO+HO \qquad (17)$$

Hydroxyl is the most reactive trace species in the troposphere and is therefore the dominant scavenger of many anthropogenic substances such as CO, hydrocarbons and halocarbons containing C—H and C═C linkages. Its concentration in the unpolluted troposphere is so low that it has proved very difficult to measure, but it is currently believed to be about 3 parts in 10^{14} at ground level, which may be compared with a value of 3 in 10^{10} in the stratosphere above 40 km where it contributes significantly to ozone removal.

Reaction with HO gives estimated tropospheric lifetimes of 2 weeks and 10 yr respectively for the important solvent molecules $CHCl{=}CCl_2$ and $CH_3.CCl_3$, which is progressively replacing it. In fact measurement of the atmospheric concentration of a compound such as $CH_3.CCl_3$ with well-known and wholly anthropogenic sources may well provide the best method for determining the average concentration of HO in the troposphere and thus its ability to remove other species from the lower atmosphere. CCl_4 is, like $CFCl_3$ and CF_2Cl_2, present in the troposphere at the 1–2 parts in 10^{10} level[13], being also unreactive towards HO. There is no obvious natural source or tropospheric removal process for CCl_4 and, as this substance is now mainly used as a chemical intermediate, it is plausible that the present quantities in the atmosphere have persisted from the time when it was used extensively as a solvent.

In an article of this length it is only possible to touch on a limited number of the topics of current interest in atmospheric chemistry. Such important but complex problems as the evolution of the Earth's atmosphere and natural cycles of such species as nitrous oxide have been omitted in favour of areas on which there have been particular emphasis in the past few years.

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Solar–terrestrial influences on weather and climate

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During the past century over 1,000 articles have been published claiming or refuting a correlation between some aspect of solar activity and some feature of terrestrial weather or climate. Nevertheless, the sense of progress that should attend such an outpouring of 'results' has been absent for most of this period. The problem all along has been to separate a suspected Sun–weather signal from the characteristically noisy background of both systems. The present decade may be witnessing the first evidence of progress in this field. Three independent investigations have revealed what seem to be well resolved Sun–weather signals, although it is still too early to have unreserved confidence in all cases. The three correlations are between terrestrial climate and Maunder Minimum-type solar activity variations, a regional drought cycle and the 22-yr solar magnetic cycle, and winter hemisphere atmospheric circulation and passages by the Earth of solar sector boundaries in the solar wind. The apparent emergence of clear Sun–weather signals stimulated numerous searches for underlying physical causal links.

ACTIVE interest in the field of Sun–weather relationships is probably higher at the present time than it has ever been in the past. Since 1972 there have been six major national or international conferences on the subject. The literature is correspondingly very large, and readers interested in going beyond what will be emphasised in this short review should consult the proceedings that have (or will have) emerged from some of these conferences[1–3], and also the extensive references contained in a recent review by Pittock[4].

Solar variability

The subject of Sun–weather relationships is built out of correlations, real or apparent, between changes in solar activity and changes in terrestrial weather and climate. No matter how variable might be the terrestrial atmosphere, if the Sun did not vary, there would be no such subject. It is therefore useful to begin with a brief account of solar variations. These fall naturally into distinct time divisions, the most familiar of which is the 11-yr sunspot cycle. There is also a 22-yr oscillation in the magnetic polarity of the leading member of sunspot pairs, the so-called Hale magnetic cycle. A longer variation of very roughly 80 yr, referred to as the Wolf–Gleissberg cycle, is seen in sunspot cycle amplitudes, as measured for example by the annual mean sunspot number.

On an even longer timescale, there is an important, recently rediscovered kind of solar variation which is typified by the now-famous Maunder Minimum, an interval in the history of solar activity extending approximately from AD 1645 to 1715 (ref. 5) The Maunder Minimum is remarkable for the near-complete absence of spots on the face of the Sun, and a concurrent absence from terrestrial skies of the aurora, a good local indicator of solar activity. It is now generally believed that ^{14}C anomalies detected in tree rings of known, specific ages mark times in the past when other weak solar activity episodes have occurred[6,7]. Episodes of anomalously strong solar activity are indicated as well. The intervals between anomalies vary, but are perhaps characteristically around 400 yr There is also a hint of a slower variation with a period around 2,500 yr associated with clustering of anomalies of the same sign.

On timescales less than the 11-yr period of the sunspot cycle, there are annual and semi-annual variations in geomagnetic and auroral activity resulting from orbital variations in the efficiency with which solar activity energises the Earth's magnetosphere. On still shorter timescales, there is the 27-d period of the solar

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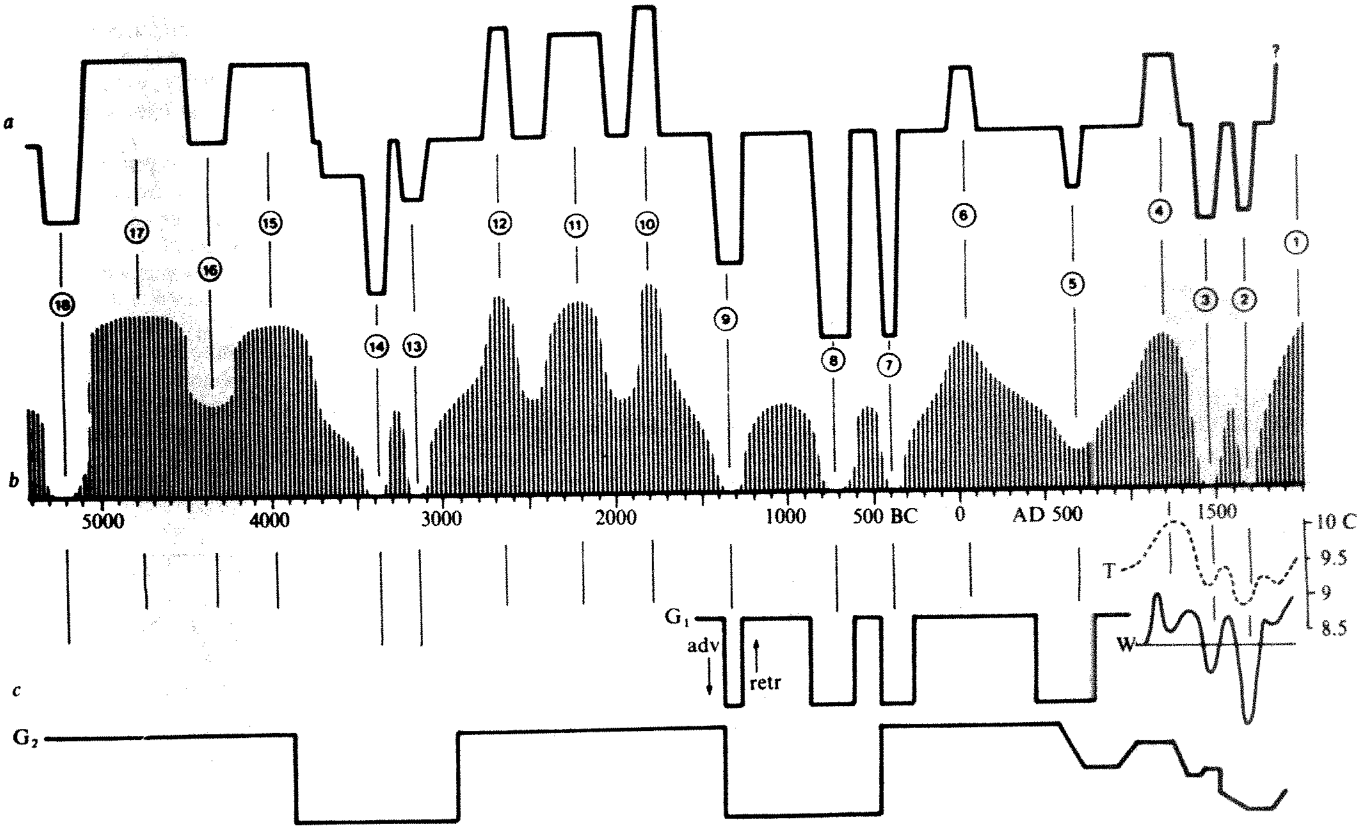

Fig. 1 From Eddy[7]. *a*, Persistent deviations in ^{14}C plotted schematically and normalised to feature 2 (Maunder Minimum); downward excursions refer to increased relative ^{14}C and imply decreased solar activity. *b*, Interpretation of curve *a* as a long-term envelope (of possible sunspot cycle) that minimises in features 2, 3, and so on, and maximises at 4, 6, and so on. *c*, Four estimates of past climate. Step-curve G_1: times of advance and retreat of Alpine glaciers, after Le Roy Ladurie[16]. Curve G_2: same for worldwide glacier fluctuations, from Denton and Karlen[17]. Curve T: estimate of mean annual temperature in England (scale at right), after Lamb[18]. Curve W: winter-severity index for Paris–London area, from Lamb[19], downward is colder.

rotation, and the variable, but approximately 8-d spacings between passages by the Earth of solar sector boundaries. In addition, at irregular intervals, solar flares produce geomagnetic storms which persist typically for 1–2 d.

Terrestrial atmospheric responses to all of the solar activity variation types listed above have been claimed or suggested, with the exception of the annual and semi-annual variations which would be impossible to detect against the background of the yearly succession of the seasons. A panel composed of participants at the symposium held recently at Ohio State University came to the historically unprecedented conclusion that three of the claimed correlations must now be granted the status of reasonable to virtual certainty. These three, correlations with Maunder Minimum-type episodes, the Hale magnetic cycle, and sector boundary passages, will be emphasised in the limited space available here. Other possible correlations, such as with the sunspot cycle and with the solar rotation, however, retain the controversial status that has traditionally characterised claims of Sun–weather correlations.

Maunder Minimum-type episodes

Atmospheric responses to the kind of changes in the Sun for which the Maunder Minimum is archetypal are of the clearest sort, global changes in climate[5–7]. The Maunder Minimum itself together with the preceding Spörer Minimum (AD 1420–1570) coincide with the two coldest excursions within the Little Ice Age, a period encompassing approximately the fifteenth to seventeenth centuries, when the surface temperatures in Europe and America were depressed by 0.5–1°C. In the case of the Maunder Minimum, the climatic event is linked directly to anaomalous solar behaviour through an ample record of telescopic observations of the Sun covering the period in question. Earlier climatic excursions, such as the Spörer Minimum and the Mediaeval Climatic Optimum, a globally warm period during the twelfth century, are linked to solar changes through proxy data. Foremost among these in importance is the tree-ring record of ^{14}C anomalies, which are believed to result when a change in the level of solar activity varies the flux of ^{14}C-producing galactic cosmic rays that can penetrate the Solar System to Earth's orbit. High solar activity reduces the galactic cosmic ray flux at Earth, which depresses the rate of ^{14}C production. The opposite condition characterises periods of attenuated solar activity. When the curve of tree ring ^{14}C anomalies is compared with the curve of climatic variations, in the words of J. A. Eddy, who has convincingly promoted and applied the field of long-term solar changes, "the correspondence, feature for feature, is almost the fit of a key in a lock" (see Fig. 1)[7].

Before the ^{14}C studies, the same long-term Sun–climate correlation had been recognised by J. R. Bray[8,9], using the solar activity index constructed by J. D Schove[10] from historical observations of aurorae and naked-eye sunspots. A more recent and more extensive compilation of historical aurorae which combines occidental and oriental observations also shows the existence of major solar activity features corresponding to the major climatic excursions[11]. Although the presently known auroral record becomes too fragmentary before the first few centuries of the Christian Era to identify solar activity episodes of the Maunder type, it may serve to validate for that purpose the use of the much longer record of tree-ring ^{14}C anomalies.

Hale magnetic cycle

The correlation that has been found linking atmospheric behaviour and the (approximately) 22-yr solar magnetic cycle involves a modulation of regional climate. The Great American Desert, which is centred roughly in the southwestern US and northern Mexico, has long been known to expand and contract its area of influence with a timescale of about 20 yr producing cycles of drought in the region west of the Mississippi River. The 'dust bowl' of the 1930s and the droughts of the 1950s and 1970s are the most recent of its intensifications and expansions. Many people have suspected and presented evidence to show that the drought cycle is related to the Hale double sunspot cycle[12–14]. Until recently the record length was too short and the drought cycle was not quantified, so that statistical tests of the proposed correlations were difficult to perform.

Mitchell and Stockton have now succeeded in quantifying the temporal climatic behaviour of the desert with a single parameter, yearly index extending back to at least AD 1700 constructed out of tree-ring indices from 40 regionally distributed, climatically sensitive sites[15]. (Actually three families with four sets of such indices were generated to establish internal consistency of the procedure.)

Power spectral analyses of the obtained data sets, so-called drought area indices (DAI), revealed well defined concentrations of power at periods near 22 yr, at formal significance levels ranging from 95% to more than 99%. The analyses were based on sets of 262 data points from the time interval 1700–1962, which included approximately 12 complete Hale cycles. Phase locking between the succession of Hale magnetic cycles and the cycles of drought area was demonstrated to exist at formal significance levels exceeding 99%. Thirdly, the amplitudes of the drought cycles vary from one to the next in a way that bears a strong resemblance to the envelope of the amplitudes of the Hale sunspot cycle. The linear correlation between the two data sets is statistically significant at between the 95 and 99% levels. Weak sunspot cycle amplitudes tend to correspond to minimum drought conditions, but the authors are quick to point out that the apparent correlation is not a reliable basis for operational climate prediction.

Although Pittock[4] has urged caution in accepting at face value the inferred Sun–weather correlation because "complex questions are involved in assessing the true statistical significance of the result", the Hale solar cycle–regional climate connection must be considered at least a serious possibility in view of its having survived the three levels of statistical tests. Full confidence in the result will most likely be achieved only if it is independently confirmed by other researchers who, like Mitchell and Stockton, have a critical attitude toward testing its reality. Such confirmation has been secured by the third of the cited correlations, which is described next.

Solar sector boundary passages

A line of investigation focused on the possibility that geomagnetic activity (a solar effect) may influence atmospheric circulation emerged in the 1950s (refs 20–23). It evolved in cast and sophistication under the stimulus of consistently positive findings until a rigorous statistical test of a specific form of the hypothesis recently could be made[24,25]. The crucial point was reached when two operationally specified, objective data sets were identified, one representing atmospheric circulation, and the other containing a statistically significant number of 'solar' events that could be used for the purposes of a correlation analysis.

The solar input to the correlation is a list of the times of passages by the Earth of solar wind sector boundaries. Sectors are regions in the solar wind, first recognised by Wilcox and Ness[26], that have predominantly one magnetic polarity, either toward or away from the Sun, and they are separated from adjacent sectors of opposite polarity by thin, usually well defined boundaries. Magnetic sectors and their boundaries tend to co-rotate with the Sun. Normally there are either two or four sectors at any given time. Magnetic sector boundaries have been shown to be natural time markers, that bear a temporally fixed relationship to the progression of a variety of solar wind phenomena. By mapping them back to their origins on the Sun, they become also natural space markers for solar features.

The atmospheric input to the correlation is a derived quantity called the vorticity area index (VAI) first defined by Roberts and Olson[24]. On a map of a constant pressure surface in the atmosphere (usually the 300 mbar level, but the correlation is also evident at 500 mbar), absolute vorticity contours are drawn at selected vorticity levels. The VAI is based on the portion of the map for the Northern Hemisphere north of 20° N, and is defined as the sum of the area (in square kilometres) over which the absolute vorticity exceeds $20\times10^{-5}\,s^{-1}$ plus the area over which it exceeds $24\times10^{-5}\,s^{-1}$. The index is specifically designed to measure the intensity and extent of low pressure troughs, which may roughly be thought of as large 'bad weather' systems.

Wilcox *et al.* have carried out a superposed epoch analysis on the VAI, using as zero days the times in a previously published list of solar sector boundary passages[27–32]. The results of their analysis based on 54 boundaries, reproduced in Fig. 2, shows an evidently statistically significant feature, of about a 6-d temporal width, centred approximately 1 d after the boundary passage, at which time the vorticity area index is depressed about 10% relative to adjacent values. The apparent signal was clearly evident only for the winter months and was entirely absent for the summer months.

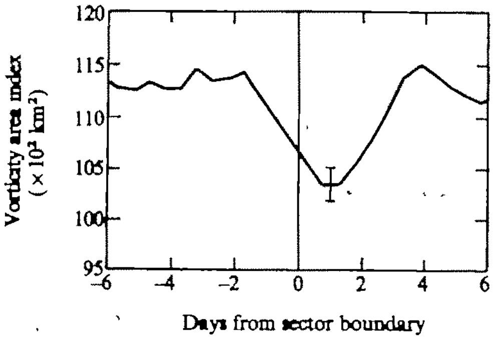

Fig. 2 From Wilcox *et al*[27]. Average response of the vorticity area index (the area of all the low pressure troughs in the Northern Hemisphere) based on the times of 54 solar magnetic sector boundary passages by Earth

The relatively short timescale of this particular claimed Sun–weather effect, less than 2 weeks, and the comparatively long interval over which VAI data were available, many years, permitted extensive testing of the statistical properties of the index over the appropriate timescale. Hines and Halevy[33,34] have undertaken the task of searching for an explanation of the alleged correlation in terms of accidental properties of the two data sets (such as having a common period of variation but derived from known, independent causes) but none was found. They ultimately concluded that either the correlation was real or it was a random fluctuation for which their analysis of the data provided a formal *a priori* probability of one chance in 20. Shapiro has carried out a similar analysis and reached the same conclusion[35]. Both critics of the claimed correlation pointed out, however, that the *a priori* probability is really not applicable because the data were in some respects selected *a posteriori*, only winter months, only a certain height interval in the atmosphere, and so on.

The issue was decided in favour of the reality of the correlation when a list of times of additional boundary passages was compiled, containing more than the original number of entries. Only if the correlation were real would one expect the signal seen in Fig. 2 to reappear in essentially identical form from a superposed epoch analysis based on the new list of times. After performing the critical test and discovering the same signal, Hines and Halevy were forced to conclude that "the alleged solar signal should be accepted as a physically meaningful signal whose origins should now be sought"[34,36].

Other correlations

Within the past century over 1,000 articles have been published asserting or refuting a claim of some form of Sun–weather influence. Most of these are concerned with correlations with the 11-yr sunspot cycle. King has published a veritable catalogue of such claimed correlations[37]. The whole field of 11- and 22-yr correlations has been critically discussed in a recent, thorough review by Pittock[4]. Multiple claims of sunspot correlations have been made for atmospheric pressure, temperature, precipitation, thunderstorms, tropopause height, and ozone, and isolated claims for an assortment of other weather–climate variations. Pittock, while reserving judgment on the new results on the drought cycle in the Western United States, concludes for the rest that "despite a massive literature on the subject, there is at present little or no convincing evidence of statistical significant or practically useful correlations between sunspot cycles and the weather or climate". Some advocates in the Sun–weather community are likely to disagree.

When statistical tools are needed to determine the reality of a correlation, especially a non-predictable one, absolute certainty will never be achieved. Instead, levels of conviction are achieved, and for the same statistical data, these vary between individuals according to whether they are advocates, agnostics or critics. Pittock's conclusion tells us that there is not yet in the field of sunspot–weather relations a single correlation with enough statistical punch to convince a critic of its reality.

It might turn out that some of the correlations are real, but a common problem that weakens the statistical approach is the shortness of the atmospheric data record compared to a signal period of 11 or 22 yr. In some cases it may be possible to extend the data record, for example through the use of proxy data, as was done for the drought cycle, with a resultant considerable increase in the strength of the statistical evidence.

As an alternative solution to the short data record problem, correlation seekers have been advised by Roberts[38] to concentrate more on short-term responses of the atmosphere to solar activity variations. The sector boundary correlation with the vorticity area index stands as a model of what can be achieved in this way. Geomagnetic and solar surface variables have been found to correlate with the VAI as well[39]. Other short-term correlations have already been suggested or claimed, such as between sunspots and quasi-biennial variations in the stratosphere[40], between the solar rotation and large amplitude standing planetary waves in the atmosphere[41], and between geomagnetic activity and a behaviour characteristic of persistence in the sea-level pressure distribution[42]. These have yet to be subjected to critical independent review.

One recently claimed short-term correlation needs to be mentioned. Attempts based on correlations with the 11-yr sunspot cycle to determine if there is a variation of global ozone with solar activity have been inconclusive. Keating[43], following a suggestion of London and Oltmans[44], has correlated monthly averaged variations of global ozone, determined from a remote sensing detector aboard the Nimbus 4 satellite, with solar activity as measured by the monthly mean 10.7 cm solar electromagnetic flux. A correlation exceeding the 95% level of confidence was found, although only 10 months of data were available for the study. The relative amplitude of the ozone variation over the study period was slightly greater than 0.1% compared with the average.

The possibility that some property of atmospheric ozone may be influenced by solar activity, which now needs to be independently confirmed or refuted with different data sets, is especially important because of the central role that ozone modulations play in current thinking about mechanisms for Sun–weather connections. It seems clear that the subject of Sun–weather connections will have the appearance of an underdeveloped science until at least one physical causal link (or one complete chain of causal links) has been verified. The determination of operating physical mechanisms would then provide a powerful means, for example, through inclusion in numerical climate models, to answer questions about the solar activity influences on specific weather–climate behaviour.

Mechanisms

It is generally recognised that there may be more than one cause and more than one effect involved in the total atmospheric response to the complex of variations that make up solar activity. The most obvious possibility, solar activity-related variation in the solar constant which powers the weather machine, remains an attractive possibility. This is true especially for the longer-term correlations, even though a recent assessment concludes that the solar constant has remained unchanged to within 0.75% over the last half of the previous solar cycle[45]. An analysis by Volland finds that planetary-scale pressure waves driven by a 0.1% 27-d oscillation of the solar constant would have detectable amplitudes[46,47]. In view of the simple direct consequences of small variations in the solar constant, there is a consensus that long-term, high-precision measurements of it would be very desirable.

Regarding Maunder Minimum-type variations, Eddy points out again that the simplest and most straightforward explanation is that solar behaviour includes a series of long-term changes in the solar constant, in this case with an amplitude of the order of perhaps 1% (refs 6 and 7). In his view, solar constant variations modulate the envelope of sunspot cycle amplitudes, accounting for the observed correlations with solar activity.

Other possibilities for explaining long-term correlations nevertheless exist. Changes in the thermal structure of the stratosphere are shown by Bates to be capable of significantly affecting the ability of the lower atmosphere to transport heat from the Equator to the Pole[48]. The thermal structure of the stratosphere can be influenced by solar activity through its modulation of solar ultraviolet radiation, which is stopped in the stratosphere[49,50], and through its supposed modulation of stratospheric ozone, which absorbs part of the solar ultraviolet. Another avenue of influence connects the same solar activity modulation of galactic cosmic rays that produces the ^{14}C anomalies in tree rings with the tendency of such cosmic rays to deplete ozone in the stratosphere, indirectly through a chain of stratospheric chemistry[51,52].

In spite of these promising leads concerning long-term correlations, it seems fairly obvious that explanations in terms of variations of one or another solar electromagnetic flux will not account for the VAI correlation with sector boundary passages. If we adopt the position expressed by Hines and Halevy, that it is time to search for the mechanisms behind this correlation, we are faced with the prospect that the responsible agents are probably part of the complex of solar particle fluxes, including the solar wind and solar cosmic rays; in which case the complete story of causal linkages will have to include the interaction of these particles with Earth's magnetosphere, and the subsequent interaction of the magnetosphere with the atmosphere.

Investigators attempting to discover such linkages have been adequately warned that the solar particle fluxes contain negligible energy compared to weather systems with which they are supposedly correlated[53,54]. On the other hand two studies show that the sector boundary–VAI correlation does not involve a detectable change in atmospheric energetics[55,56]. The situation thus favours the kinematical explanation offered by Hines and Halevy, in which phase shifting of pre-existing meteorological noise is invoked to produce the observed coherence[33,34]. The required phase-shift mechanism, however, has not yet been identified.

Present exploration aimed at locating a coupling mechanism that can convert a solar wind stimulus into an atmospheric response on a short timescale is focusing on the magnetospheric particles that precipitate into the upper atmosphere, and which are known to be modulated by the solar wind. Examples are the precipitations that produce the polar aurorae[57], and the rarer but more penetrating "relativistic electron precipitation" (REP) events[58]. Some argue that these may act, for example, through promoting the development of cirrus clouds high in the troposphere[24] or by modifying the elements in the global atmospheric electrical circuit, thereby possibly affecting thunderstorm activity[59].

This short list of ideas is not intended to show that the problem of identifying the causal links connecting solar activity and weather or climate is close to being solved. On the other hand, now that some of the claimed correlations have risen respectably high out of the noise, the problem is receiving increasingly more attention. There is reason for optimism that the next landmark in the field of Sun–weather relationships, the verification of a causal link, may not be far off.

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Predictability of climate

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A mathematical framework is presented for the study of the predictability of small changes in the climatic system. From a statistical definition of climate in terms of ensemble averages, a limit to predictability is found arising from random weather fluctuations. This limit, typically referred to as climate noise, is often able to obscure a possible climate signal produced by external forcing. A separation of timescales of climatic response is also presented as a convenient means to distinguish between the fast internal (for example, the atmosphere) and the slow external (for example, the oceans) systems. In this way, it is possible to estimate the climate signal in the internal system that arises from small changes in the slow external system. Finally, a theoretical approach is presented for the inclusion of possible feedback effects in the estimation of a climate signal. Such feedback effects could result from the external climatic system being influenced by a change in the mean properties of the fast internal climatic system.

In recent years a number of widely publicised anomalies in various parts of the world have greatly increased interest in the predictability of climate for periods of months to decades. In this review I shall define climate, as distinguished from weather, in terms precise enough to serve as a basis for a statistical dynamical theory of small fluctuations. The problem is to separate potentially predictable fluctuations called signals from unpredictable fluctuations called noise. The ratio of signal to noise provides a measure of the predictability of the climate system.

Definition of climate

The climate at any place is commonly defined as being the average weather there. A broader definition of the global climatic system includes all the statistical properties of the global atmosphere and the underlying land, sea and ice surfaces. The climate system is thus described in terms not only of mean values but also of variances about the mean, co-variances between variables possibly separated in space and time, probabilities of extreme events, and so on. Specification of the statistical properties of the climatic system involves far more information than does specification of the state of the atmosphere at a given time, but little is known about the statistical moments of the system higher than the second.

The statistical properties of the climate system must be defined in terms of some sort of averaging process and here fundamental difficulties arise. The common, operationally feasible, and natural choice is to compute an average over some time interval T. This permits a continuum of choices depending on T. For small enough values of T the corresponding time-averaged climate would become indistinguishable in its fluctuations from the weather. For greater and greater values of T the corresponding time-averaged climate becomes increasingly

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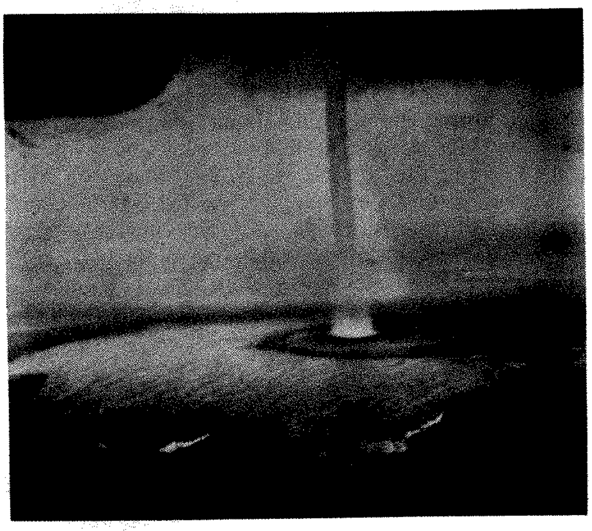
independent of the averaging interval, and we might define a true climate in terms of a limit as T approaches infinity. In addition to the practical difficulty that computing an infinite time average is no longer operationally feasible, there is the major theoretical difficulty that we would now no longer be able to consider climate as changing with time. A conventional compromise is to take T as 30 yr.

Such a compromise definition of climate is awkward for setting up a precise mathematical framework for a theory of climate. This same general difficulty was encountered over a century ago in considering the formulation of a statistical mechanical basis for thermodynamics. In this analogy the detailed motions of gas molecules correspond to the weather and the statistical properties of a gas such as temperature and pressure correspond to the climate. Here too, if one wishes to discuss a situation in which temperature and pressure are changing, one must not define them in terms of infinite time averages.



Waterspout photographed near Florida Keys, 10 September 1969, by J. H. Golden of ERL-NOAA, Boulder, Colorado (note the spiral forming on the surface of the water)

In statistical mechanics this difficulty has been circumvented by introducing the theoretical concept of an ensemble. An ensemble is an essentially infinite collection of copies of the original dynamical system with each evolving in its own natural way. The dynamical state for each copy is specified by giving the values of a large number of dynamical variables, and it can therefore be represented by a point in a multi-dimensional dynamical phase space. At any given time the members of the ensemble will be found at phase points throughout the whole phase space in accordance with some probability distribution. In time the phase points move and thus in general the probability distribution changes. The problem in statistical mechanics is to find an equilibrium probability distribution that does not change with the natural motion of the phase points in order to define statistical properties in terms of the corresponding ensemble average. One may then ask sensible questions about how external influences can modify statistical properties by changing the equilibrium ensemble probability distribution.

The application of this general concept to the climate system is straightforward. One imagines an ensemble of Earths, each subjected to the same external influences, each with the same distribution of continents and oceans, and the weather on each going through normal fluctuations independently of all others. One next imagines that the distribution of states of the weather in the ensemble is chosen in such a way that the probability distribution is unaffected by the rapid weather fluctuations of individual members but can change relatively slowly owing to changing external influences. The climate is then defined in terms of averages over this ensemble. It is immediately evident that this ensemble-averaged climate is an ideal theoretical concept inaccessible by direct observation just as was the infinite time-averaged climate discussed previously. For a system with unchanging external influences, it is desirable for the two definitions to be equivalent. A dynamical system is said to be ergodic or transitive if this is the case.

At this point the objection might be raised that the ensemble-averaged climate is not really what anyone is interested in. We live on only one Earth with a single sequence of weather events and are naturally more concerned about the future behaviour of that single system than in some average behaviour of an imagined ensemble. Accordingly, the most direct way to predict the climate would seem to be to predict the weather and then to average it in time. Unfortunately, we are currently unable to predict most of the variability of the weather beyond a few days. Furthermore, theoretical studies indicate that there exists a limit of a week or so in the range of useful prediction even for a perfect prediction model because of the unstable nature of the atmosphere and the inevitable inadequacies in observations of its detailed initial state.

We adopt then the point of view that the climate is determined by an ideal ensemble and that finite time averages will fluctuate about ensemble values producing what is called climate noise. This climate noise, to be discussed in the next section, arises from the statistical fluctuations of the unpredictable weather and not only obscures the properties of the true climate but also diminishes the value of knowledge of these properties for prediction of the short-term behaviour of the most interesting member of the ensemble, the one which actually exists.

A further question arises in seeking a definition of climate precise enough for research purposes. How does one bound the statistical climate system to distinguish its internal behaviour from external forcing influences? Ideally, this should be done in such a way that the external forcing influences are independent of the state of the internal system. They may be taken, for example, to be the positions of the continents and the incoming solar radiation at the top of the atmosphere. For many model studies of the climate, however, it is useful to specify as external a much larger number of influences such as carbon dioxide content, sea surface temperature, ice sheet thickness and surface albedo. Although the separation between the internal and external systems may be made in many ways, it is important to remember that any feedback influences of the internal system on the external system must either be ignored or treated in an ensemble-average sense. Otherwise, the external system acquires some of the unpredictable random behaviour of the internal system and can no longer be sharply specified.

By judicious location of the boundary between the internal and external systems, we can also implicitly separate time scales of change. The advantages sought in an explicit choice of averaging time scale can instead be obtained by choosing the internal system to include all relatively fast processes. To achieve a time scale separation at a few weeks, for example, the internal system would contain the atmospheric motions and changing land surface temperature, whereas the sea surface temperature would be treated as an external forcing influence.

Climate noise

Perhaps the most familiar example of a sampling fluctuation occurs with the tossing of a coin. Although for a large number of tosses the ratio of heads to tosses is expected to approach one-half, no-one is greatly surprised if 20 tosses result in 7 heads and 13 tails. If 200 tosses, however, were to result in 70 heads and 130 tails, some doubt might be raised as to the fairness of the coin. The theory of such fluctuations is completely understood and can be found in any elementary textbook on probability theory.

A similar example more relevant to the continuous variables of the climate system is the drawing of a sample of n independent values x from an infinite population with mean μ and standard deviation σ. The average $\bar{x}$ of the sample generally will differ from μ, and it is easy to show that many repetitions of the sampling procedure will lead to a distribution of $\bar{x}$ with mean μ and standard deviation $\sigma_n = \sigma / n^{1/2}$.

Averages of a meteorological time series over an interval T have similar fluctuations about the infinite population mean, but in this case sequential observations, if frequent enough, are not mutually independent. This may easily be accounted for[1,2] in terms of an effective time between independent samples

$$T_o = \int_{-\infty}^{\infty} R(\tau)\,d\tau$$

where $R(\tau)$ is the correlation of two values in the time series separated by a time interval τ. For $T/T_0 = n$ sufficiently large, n plays the role of the independent sample size. The standard deviation σ_T of time averages is then related to the standard deviation σ of the time series by

$$\sigma_T = \sigma / n^{1/2} = \sigma (T_0/T)^{1/2}$$

The time T_0, which is an integral time scale for fluctuations of meteorological variables, plays an important part in the determination of climate noise levels. Madden[3] has determined T_0 for sealevel pressure over much of the Northern Hemisphere and finds values with a range 2–8 d.

Tornadoes over Vietnam photographed from a helicopter. Taken from *The Elements Rage* by F. W. Lane (David and Charles, Newton Abbot, UK)

Some part of the fluctuations from year to year in monthly averages of meteorological variables must be attributed to the climate noise arising from sampling fluctuations. As such it represents an unpredictable component, and a key question for climate predictability is how much greater is the variability observed to be than the amount expected from noise. Madden[3] has attempted to answer this question for monthly average sea surface pressures for January and July. He has found that most of the observed interannual variability in midlatitudes seems to be climate noise, whereas in the tropics there is evidence of an additional potentially predictable component.

The existence of an unpredictable noise component in finite time averages limits the practical utility of any predicted shift $\Delta\mu$ in the climate mean μ which we may appropriately call a climate signal. Unless the signal-to-noise ratio $\Delta\mu/\sigma_T$ is greater than about 0.5, the prediction of $\Delta\mu$ may have little utility for prediction of time averages of duration T (ref. 1). Of course, as σ_T decreases at $T^{-1/2}$, for long enough intervals, T, the prediction of $\Delta\mu$ acquires value.

Climate signal

In the previous section I described the climate noise that arises from unpredictable weather fluctuations in the fast internal climatic system. In this section I shall discuss procedures for estimating the climate signal that arises from the influence of the slow external system on the internal system. To the extent that the slow system may be predictable, the coupling of the two systems provides an element of predictability for the fast system.

The usual procedure for determining the response of the internal system is to construct a numerical model of it in which the external influences are treated as specified forcing terms. Symbolically, we may write the model dynamical equations for the unperturbed system as

$$\dot{x}_\alpha = Q_\alpha(x_1, x_2, \ldots, x_N) + f_\alpha$$

Here $x_1, x_2, \ldots, x_N$ are amplitudes of a large but finite number N of modes in terms of which the state of the internal system is defined, $Q_\alpha(x_1, x_2, \ldots, x_N)$ are complicated functions determining the internal dynamics of the system and f_α are forcing terms describing the external influences. For the statistically stationary unperturbed system, we have unchanging ensemble means, that is, $\langle \dot{x}_\alpha \rangle = 0$. If now a step change in forcing δf_α is introduced at time $t = 0$ for all members of the ensemble, then the ensemble means will start to change at a rate $\langle \dot{x}_\alpha \rangle = \langle \delta f_\alpha \rangle$. Because of the stable nature of the climatic system, a new stationary level will be approached with a final change in means given by $\delta \langle x_\alpha \rangle$.

The general procedure in using a numerical model to estimate $\delta\langle x_\alpha \rangle$ is to make long computations with and without the perturbed forcing δf_α, to estimate $\langle x_\alpha \rangle$ in either case by time averages over intervals excluding any initial transient, and to subtract one estimate from the other. The climate noise associated with the time averages tends to obscure small values of $\delta\langle x_\alpha \rangle$. It is usually assumed that small responses are linear and that δf_α may be amplified by a scale factor by which the computed response is later divided.

The first-order linear response of the system can be written as

$$\delta\langle x_\alpha \rangle = \sum_\beta A_{\alpha\beta}\, \delta f_\beta$$

where the matrix elements $A_{\alpha\beta}$, having the dimensions of time, determine the response of the mean of any mode $\langle x_\alpha \rangle$ to a change in forcing of any mode δf_β.

Another procedure provides more detailed information about the time history of the response. Consider an impulsive forcing which is turned off immediately after being imposed at time $t = 0$ on each member of the ensemble. This produces an immediate change $\delta\langle x_\alpha(0^+) \rangle$ which will then tend to zero as the system returns to its original statistical state. For small perturbations the relaxation is described by

$$\delta\langle x_\alpha(\tau) \rangle = \sum_\beta g_{\alpha\beta}(\tau)\, \delta\langle x_\beta(0^+) \rangle$$

where $g_{\alpha\beta}(\tau)$ is a mean linear response matrix function of time τ with $g_{\alpha\beta}(0^+) = \delta_{\alpha\beta}$ the identity matrix. The response to any history of forcing perturbation $\delta f_\alpha(t)$ may be written in terms of $g_{\alpha\beta}(\tau)$ as

$$\delta\langle x_\alpha(t) \rangle = \sum_\beta \int_{-\infty}^{t} g_{\alpha\beta}\,(t-s)\, \delta f_\beta(s)\, ds$$

In particular, for step forcing the infinite time response matrix is given by

$$A_{\alpha\beta} = \int_0^\infty g_{\alpha\beta}(t)\, dt$$

A determination of $g_{\alpha\beta}(\tau)$ from model calculations is probably not feasible, but it can be estimated by statistical analysis of the unperturbed climate system itself. In the course of their natural evolution, the members of the ensemble generate anomalies δx_α from the mean. For a sub-ensemble all of whose members have a given anomaly $\delta x_\alpha(0)$ at time $t = 0$, the sub-ensemble mean perturbation will be $\delta\langle x_\alpha(0) \rangle = \delta x_\alpha(0)$. But it, too, will tend to return to zero in a way that can be estimated by linear regression prediction methods.

The timelagged co-variance matrix for the unperturbed system is

$$X_{\alpha\beta}(\tau)=\langle\delta x_\alpha(t+\tau)\,\delta x_\beta(t)\rangle$$

the regression matrix is

$$R_{\alpha\beta}(\tau)=\sum_\gamma X_{\alpha\gamma}(\tau)\,[X^{-1}(0)]_{\gamma\beta}$$

where $R_{\alpha\beta}(0)=\delta_{\alpha\beta}$, the identity matrix, and the least square estimate of the future mean anomaly of the sub-ensemble is given by

$$\delta\langle x_\alpha(\tau)\rangle=\sum_\beta R_{\alpha\beta}(\tau)\langle\delta x_\beta(0)\rangle$$

The reasonable assumption that the system recovers from a small natural anomaly in the same way that it does from one induced by external forcing leads to the fluctuation dissipation relationship that for $\tau\geqslant 0$

$$g_{\alpha\beta}(\tau)=R_{\alpha\beta}(\tau)$$

According to the fluctuation dissipation theorem$^{4-7}$, this relationship holds exactly for dynamical systems with a gaussian Maxwell–Boltzmann equilibrium probability distribution. Such is not the case for a dissipative turbulent system such as the atmosphere, but experience with turbulence models has indicated that the approximation may nonetheless be fairly good.

One is faced with the choice of estimating $g_{\alpha\beta}(\tau)$ for the internal climatic system either as given by $R_{\alpha\beta}(\tau)$ for the system itself or as given by $g_{\alpha\beta}(\tau)$ for a numerical model of it. Confidence in the results would be greatly enhanced if the two quite different approaches gave similar answers, and this confidence would extend to model results for perturbations beyond the linear domain of the fluctuation dissipation relationship.

Qualitative properties of climate signals may be deduced from known qualitative properties of correlations using the fluctuation dissipation relationship, as regression functions are qualitatively like correlation functions. These have a spatial extent in midlatitudes of a few thousand kilometres, and the climate response to a local forcing perturbation should be similarly limited in spatial extent. This suggests that the climatic system may be considered as a collection of more or less independent regional climatic systems.

As the diagonal regression coefficients $R_{\alpha\alpha}(\tau)$ are qualitatively like the single variable lagged correlation function $R(\tau)$ discussed in the previous section, a qualitative estimate of the step response coefficients for the fast internal system is given by

$$A_{\alpha\alpha}=\int_0^\infty R_{\alpha\alpha}(\tau)\,d\tau\approx T_0/2\approx 3\text{ days}$$

The internal climatic system adjusts therefore within a week or so to a new level with a change in mean values $\delta\langle x_\alpha\rangle$ given by the initial rate of change δf_α multiplied by a characteristic response time of a few days.

Feedback effects

It has been assumed that the external climatic system is not influenced by the mean or fluctuating properties of the fast internal climatic system. The feedback influence of mean effects, however, may be included rather easily to first order by linear analysis. It is convenient here to replace matrix elements by matrix notation so that the step forcing response equation becomes

$$\delta\langle\mathbf{x}\rangle=\mathbf{A}\,\delta\mathbf{f}$$

Suppose now that a change in mean properties $\delta\langle\mathbf{x}\rangle$ in the internal system forces a change in the external system leading eventually to an additional change in forcing $\delta_1\mathbf{f}$ which to first order is given by

$$\delta_1\mathbf{f}=\mathbf{B}\,\delta\langle\mathbf{x}\rangle$$

The combined effects are then given by

$$\delta\langle\mathbf{x}\rangle=\mathbf{A}(\delta\mathbf{f}+\mathbf{B}\delta\langle\mathbf{x}\rangle)$$

so that we have

$$\delta\langle\mathbf{x}\rangle=(\mathbf{I}-\mathbf{B})^{-1}\,\mathbf{A}\,\delta\mathbf{f}$$

The feedback effect matrix $(\mathbf{I}-\mathbf{B})^{-1}$ can either increase or decrease the response $\delta\langle\mathbf{x}\rangle$ from that with $\mathbf{B}=0$.

The response of the external system is assumed to be slow and, for this present analysis, non-random. The response properties leading to the matrix $\mathbf{B}$ may therefore be determined relatively easily using a numerical model of the external system that includes the appropriate slow physical processes.

As with the internal system, it is possible to determine the more detailed time history of the feedback response of the external system. Qualitatively, a step change $\delta\mathbf{f}$ induces a rapid change $\delta_0\langle\mathbf{x}\rangle$ which induces a slow change $\delta_1\mathbf{f}$ which induces a further slow change $\delta_1\langle\mathbf{x}\rangle$ to the final value $\delta\langle\mathbf{x}\rangle=\delta_0\langle\mathbf{x}\rangle+\delta_1\langle\mathbf{x}\rangle$ given above.

The additional feedback influence of the random fluctuations in the internal system has been discussed by Hasselmann^8 and others^{9,10}. Now the external system becomes random and Hasselmann considers one that can be modelled by a Langevin equation of the general form

$$\dot{\mathbf{y}}=-\mathbf{D}\mathbf{y}+\mathbf{g}$$

Here $\mathbf{y}$ represents anomalies in the external climatic system, $\mathbf{D}$ is a matrix which is positive definite to provide stability and $\mathbf{g}$ is a random white forcing term that models the effects of the rapid fluctuations of the internal system. The resulting behaviour is analogous to damped brownian motion with the external system playing the part of the particle and the internal system that of the molecular motions. By suitable choice of $\mathbf{D}$ and the strength of $\mathbf{g}$, it has been possible to model the frequency spectrum of sea surface temperature fluctuations considered as part of the external system^{9,10}. Some predictability of the external system is provided for the Langevin models by the mean regression equation

$$\langle\dot{\mathbf{y}}\rangle=-\mathbf{D}\langle\mathbf{y}\rangle$$

Discussion

In this review I have described the mathematical framework for the study of the predictability of those small changes in the climate for which a linear analysis is appropriate. For larger changes a non-linear numerical climate model must be used.

Much work has yet to be done to fill in this framework. There is already fair evidence for useful climate predictability in equatorial and tropical regions where the noise level is small, but there is only fragmentary evidence for it in midlatitudes.

Finally, the possibility of extending the range of deterministic prediction of relatively stable internal configurations of the sort that arise in atmospheric blocking events is of great practical importance. But this would be a prediction of weather rather than of climate as strictly defined in this review.

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Predicting temperature trend in the Northern Hemisphere to the year 2000

M. K. Miles*

The cooling of the Northern Hemisphere since 1940 has been variously interpreted as the overture to the next Ice Age, the effect of industrial pollution in the atmosphere or of a decline in the solar output. Are we in a position to judge between these various interpretations and to make a prediction for the next few decades? The link with the next Ice Age may be dismissed as a confusion of timescales; the explanation in terms of atmospheric pollution merits careful examination but seems unlikely to be adequate on its own. Natural fluctuations must also be considered.

THE annual surface temperatures of the Northern Hemisphere from 1951 to 1975 given by Kukla *et al.*[1] show that year to year differences equalled or exceeded 0.3 °C eight times in this period. These changes do not constitute climate as generally understood, although they may be very important for world agriculture or transportation. There are various views on how long a period we should average this temperature over to arrive at the value which is to be the subject of climatic predictions.

How 'noisy' is the temperature record?

The data themselves do not give us much guidance: variations seem to occur on all time scales from a year to millennia. Claims have been made at various times that at some particular frequency (2.2 yr, 11 yr, 88 yr, 200 yr, and so on) there is a marked periodicity, but the variability associated with any of these is usually a small part of the total: no period stands out so clearly from the rest that we can on this basis select an appropriate averaging period.

The spectrum is not, however, simply a 'white noise' one with all frequencies equally represented: there is a notable tendency for there to be more variation at periods above about 10 or 20 years than a white noise spectrum would contain. This I interpret as meaning that it may not be a fruitless task to try and say something about the future trend of means taken over 5 or, perhaps better, 10 yr.

Figure 1 shows the course of decadal means of Northern Hemisphere surface temperatures for the past 100 yr. These have a standard deviation of 0.17 °C but the deviations are not randomly distributed. A random series with this standard deviation would show average decade to decade differences of about 0.24 °C, whereas this series has an average difference of 0.11 °C between adjacent decades. Moreover, since 1915 there have been three adjacent positive values (that is, a warming trend) followed by three successive negative values—a pattern which hints strongly at a dominance of non-random effects.

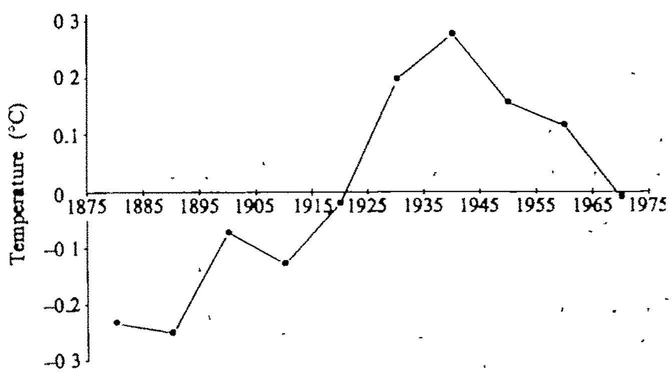
If we suppose for the moment that a warming trend of 0.1 °C per decade from 1885–1944 followed by a cooling trend of the same magnitude to 1974 represent non-random effects, then the decadal values have a random fluctuation corresponding to a standard deviation of 0·05 °C superimposed on this pattern. This is certainly a minimum value of the 'noise', compounded of instrumental errors and natural fluctuations not taken into account. As most explanations do not account for more than about two-thirds of the variance over the past 100 yr, a better working estimate for the standard deviation of the noise is probably 0.1 °C. This means that there is a 5% chance that the next decade (1975–84) will be ± 0.2 °C or more different from 1965–74 due to 'noise'. Thus, a continuance of, or reversal of, the trend could not reliably be inferred from such a change. If, however, the following decade showed a further 0.1 °C or more movement in the same direction this could be considered as an organised trend with a high probability that it was not due to chance.

From such considerations one could set up three reasonable categories for predictions of the decade centred around AD 2000: (1) 0 3 °C or more above the value for 1965–74, implying a reversal of the cooling trend of the past 30 yr with temperature at least back to that of the optimum in Fig. 1; (2) 0.3 °C or more below the value for 1965–74, implying a continuance of the cooling trend at about the same rate, and (3) within ± 0.3 °C of 1965–74 which implies an expectation of random fluctuations about the value of the latest decade.

Some predictions or quasi-predictions

The published predictions are not always quantified; they tend to say either that the cooling trend will continue or that it will reverse. Among those favouring continued cooling, Lamb has sometimes mentioned a return to the conditions prevailing at the end of the eighteenth century, which would put his forecast in category (2); and Bryson[3] is such an enthusiastic advocate of cooling that his can hardly be put in category (3). Budyko and Vinnikov[4], the most emphatic advocates of a warming, predicted a value for AD 2000 of between 0.5 and 1.0 °C above the latest decade—a prediction clearly in category (1). Mitchell[5] expected that the warming effect of CO_2 and the cooling effect of man-made dust were likely to be in near balance to the end of the century. One might deduce from this that natural fluctuations would determine the sign of the deviation. His expectation could probably be assigned to category (3), but he considers a specific prediction to be unwarranted. Kellogg[6] likewise thinks that we are not in a position to make a categorical forecast: he considers that a warming of 0.5–1.0 °C due to increasing CO_2 is fairly likely by AD 2000, whereas cooling due to man-made aerosol is more doubtful and is likely to be less than this. Thus, he concludes that man's activity will probably raise Northern Hemisphere temperature by some 0.5 °C by the end of the century and natural fluctuations are unlikely to mask this warming trend.

Fig. 1 Decadal means of surface temperature for the Northern Hemisphere, plotted as deviations from the average, 1875–1974 (After Budyko, Willett and Mitchell to 1969; updated to 1974 from Kukla *et al.*[1])



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Rationale of the predictions

Budyko and Vinnikov[4] and Bryson[3] interpret the temperature trends over the past 100 yr in terms of the changing transmissivity of the atmosphere. They base this on a Russian time series of direct radiation measurements first assembled by Pivovarova in 1968 and later updated by Budyko and Vinnikov[7]. They are shown in Fig. 2 as deviations in percentage from the average for 1885–1970. The data for the early decades come mainly from Russian observatories but after about 1914 data from American observatories are included. There is an increase of about 4% in direct radiation between the turn of the century and 1940 followed by a decrease of about 6% by the late 1960s. Budyko and Vinnikov[4] and Bryson[3] attribute the increase mainly to the clearing of the atmosphere following the series of violent volcanic eruptions in the 1880s, and the decrease after 1940 to the increased concentration of man-made dust due to industrial activity. From observations of the ratio of scattered to direct radiation at the surface Budyko and Vinnikov[7] conclude that the 4% gain of direct radiation implies a net gain of something under 1% (there is less scattered radiation) which is more than enough to explain the 0.6 °C rise in the Northern Hemisphere temperature over that period.

On the other hand there is considerable doubt whether enough of the volcanic dust ejected into the stratosphere by the Alaskan eruption in 1912 (Katmai)—the last major eruption until the 1950s—would be still present even 10 yr later to depress the temperature noticeably below a clear-air value. Yet about half of the increase in temperature occurred after that date, and Miles and Gildersleeves[8] have argued that this cannot be attributed to the clearing of volcanic dust. Although there is considerable evidence that dust after major eruptions depresses the hemisphere temperature by 0.2–0.3 °C for about 2 yr afterwards, the effect of a single major eruption on the decadal temperature is not likely to exceed 0.15 °C.

The series of major eruptions around the time of Krakatoa (1883) may have depressed the temperature of the 1880s by up to 0.3 °C but after that there has not been a comparable series of eruptions. The Russian series of direct radiation measurements shown in Fig. 2 suggests that the radiation recovered to a normal level after the eruptions of the 1880s, after those of 1902–03 and the Alaskan eruption in 1912. Although there is still some doubt about the response time of the atmosphere, it is likely that the hemispheric temperature returned to near the clear-atmosphere value after each. Thus the depressive effect of these volcanic episodes cannot be considered additive. If this is accepted, then the magnitude of the warming due to the clearance of volcanic dust can hardly be placed higher than 0.3 °C. This leaves most of the warming after 1920 to be explained in some other way and also the 1% increase in direct radiation after 1920. It is probable, however, that the radiation series is not homogeneous, as the number of stations used was increasing with time and it is difficult to be sure that calibrations are the same within 1% over these long periods.

The interpretation of the 6% decrease after 1940 presents further difficulties. First, it has not been demonstrated beyond a doubt[9] that the average atmospheric dust loading of the Northern Hemisphere has increased. The transmissivity at some locations has not changed during this century. It seems very probable that increased industrial activity has put more dust into the lower atmosphere but it seems that the bulk of it falls out within a few hundred kilometres of the entry zone, so that the average increase in tropospheric dust over the whole hemisphere is a very small amount indeed. The reduction in direct radiation shown by the Russian series, if due to man-made dust, is therefore considered to apply only in the neighbourhood of the observatories in Eurasia and North America.

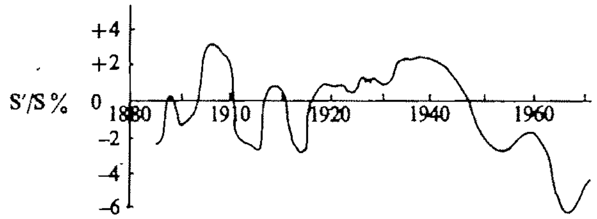


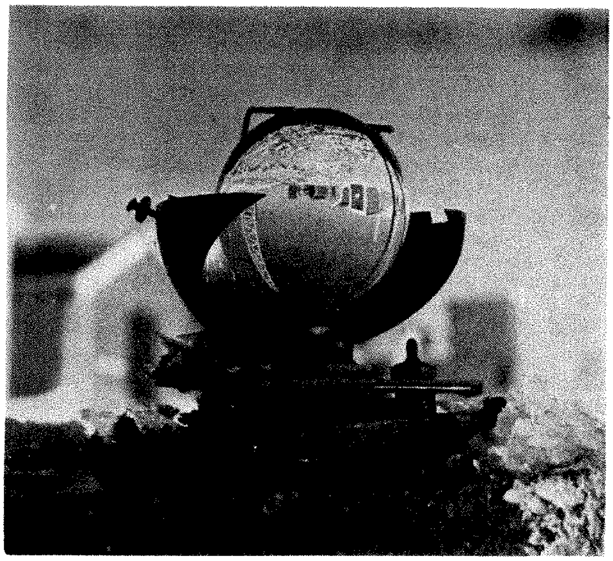
Fig. 2 Five-year means of direct solar radiation anomalies. (From Budyko and Vinnikov[7], based on work by Pivovarova.)

Second, dust absorbs solar radiation as well as scattering it, so that there is a warming effect arising from the absorption of the direct solar radiation. In the case of volcanic dust (in the stratosphere) the warming is confined to the stratosphere but if the bulk of the additional man-made dust is fairly low down in the troposphere this warming could affect the surface temperature. It is uncertain how much this will offset the loss by scattering. Budyko[4] reduces the cooling by one-third and, after making a further allowance for the fact that much of this dust will be below cloud level, concludes that this 6% reduction in direct radiation will cause a temperature reduction of 0.5 °C. This together with a warming of about 0.2 °C—a widely accepted estimate—due to the additional CO_2 gives a net cooling of the right amount. Bryson[3] considers the two effects together and arrives at a net cooling of 0.3 °C. Although the two estimates are in agreement, the uncertainties in reaching them are so great that it is important to explore other ways of assessing whether they are likely.

If the dust absorbs long-wave terrestrial radiation giving a so-called greenhouse effect then the net cooling due to the scattering effect may be very small indeed. Indeed Idso and Brazel[10] have put forward evidence that with realistic concentrations of dust the net effect on the surface temperature would be a warming. Without going this far it seems likely that the surface cooling due to a given concentration of man-made dust in the lower troposphere will be less than that due to the same concentration of volcanic dust in the stratosphere. Mitchell[5] has given estimates of the loading of man-made dust which show that at the end of the 1960s it was about half the estimated average loading of stratospheric volcanic dust in the 1880s. If the depression of Northern Hemisphere temperature below a clear-air equilibrium value for the 1880s could be reliably established, this would give an upper limit to the cooling to be expected from the recent concentration of man-made dust.

Already a divergence of opinion appears. Bryson[3] (and to some extent Budyko[4]), in saying that practically the whole of the 0.6 °C warming from the 1880s to the 1940s is due to the clearing of the volcanic dust, is putting this upper limit at 0.6 °C. Lamb[2] is inclined to the view that other factors were as important in bringing about the warming. It is difficult from the hemispheric temperature data to see how the depressive effect in the 1880s could have exceeded about 0.3 °C and if the effects of the successive eruptions are not additive this would be the upper limit to the cooling effect of the man-made dust in the late 1960s.

Agreeing as they do on the cause of the post-1940 cooling, how is it that Bryson and Budyko then reach such divergent views about the future trend? Bryson regards the cooling since 1940 as showing that the extra opacity due to industrial activity has a larger effect on surface temperature than the reduced emissivity due to the increased CO_2 arising from the same industrial activity. Thus, the expected increase in industrial activity during the rest of this century will bring a steadily decreasing surface temperature. Budyko, however, says that present pollution control measures are reducing the input of dust but not that of CO_2 and that eventually the warming effect of CO_2 will be decisive. He advances two pieces of evidence that this is already happening. The amount of direct solar radiation has, he says, increased since the low values of the 1960s, and the hemispheric temperature has risen by 0.3 °C in the past decade. The reservations concerning the radiation measurements have already been discussed and no other climatologist has claimed to find such a marked increase in temperature for the Northern Hemisphere. Most estimates indicate a reduction of the cooling rate almost to zero in the 1970s but do not show a reversal.



Campbell-Stokes sunshine recorder photographed by W. Scriven, Great Dunn Fell, Westmoreland (now Crown Copyright, Meteorological Office, Bracknell, UK)

Budyko supports his claim for a hemispheric warming by pointing to a warming of about 1.0 °C in the polar regions since the mid-1960s. This polar warming also emerges from an analysis of data from ice flow stations. It reduces the size of the meridional temperature gradient and he claims that in the past a reduction in meridional temperature gradient has been generally associated with an increase in the hemispheric temperature. There has however, been at least one period in the past (1935–44) when the polar temperature was decreasing while that of the hemisphere was increasing, and it would be advisable to have more direct evidence of a hemispheric warming before accepting Budyko's thesis.

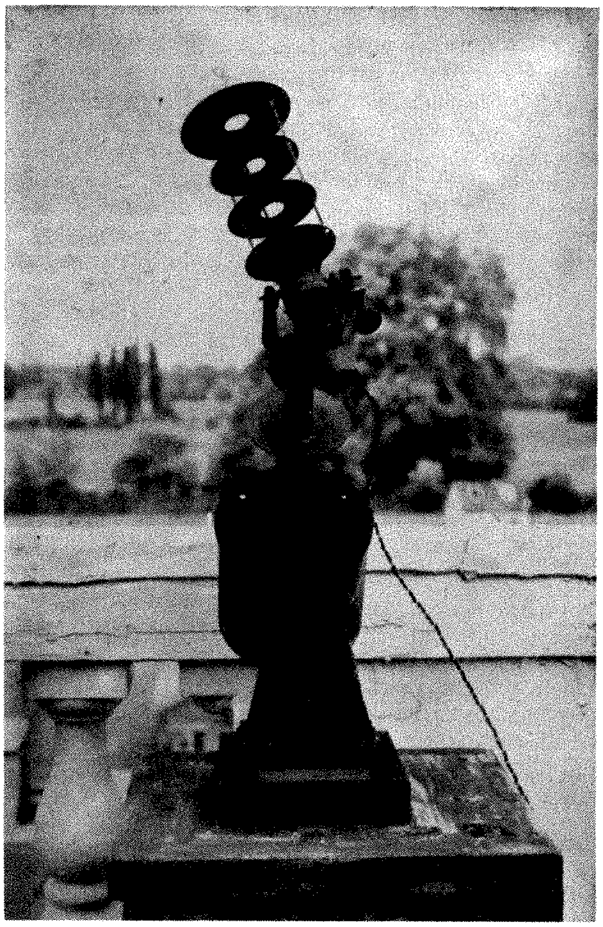
One of the secondary reasons for believing that dust has led to such a substantial cooling since 1940 is that the observed cooling has to be explained in the face of an expectation that the increased CO_2 has increased the equilibrium temperature since the turn of the century by about 0.3 °C. This is widely regarded as the one effect known most precisely but it is not entirely satisfactory to use it as indirect support for the man-made dust case. The net effect of the CO_2 with cloud feedback may be substantially less than this. Besides, if the further increase in temperature after 1920 is neither due to the clearing of volcanic dust nor to the increased CO_2 then a return to the level of the 1920s, if the causative mechanism ceased after 1940, would not be altogether surprising.

There are moreover some grounds for thinking that 0.3 °C is an overestimate of the hemispheric cooling since 1940. Sea temperature data for the tropical Atlantic and Pacific now being analysed in the UK Meteorological Office indicate that temperature increased from a minimum in the 1920s until the early 1960s, and the mean value for the 1960s is probably higher than that for the 1930s. There is some reason to think that the published estimates of Northern Hemisphere temperature (on which the graph in Fig. 1 is based) gave insufficient weight to temperatures in the tropics. If the course of the surface air temperature of the tropics followed that of the sea temperature, then the cooling since 1940 would probably be less than 0.3 °C. This perhaps strengthens the man-made dust case because one of the objections against it is the magnitude of the cooling effect claimed. On the other hand, if pollution control measures are increasing the atmospheric transmissivity as Budyko (and some others) are claiming, then the warming due to increasing CO_2 may more easily balance the cooling effect of dust and even become the dominant effect before the end of the century.

Prediction of some natural fluctuations

If these two man-made effects on climate are likely to be in near balance until the end of the century, this would leave the outcome to be determined by natural fluctuations. These may be due to variations in solar output or planetary albedo (apart from changes due to man-made dust) on the one hand and differences in the amount of heat stored in the oceans arising perhaps from variations in the general wind circulation on the other. Mitchell[5] and Kellogg[6] believe that we are not in a position to predict any of these effects. Some other climatologists are not so cautious and make a prediction of continued cooling based on indirect evidence of a change in solar output associated with long-term variations in the amplitude of the 11-yr sunspot cycle. The number of dark spots on the Sun increases from near zero to a maximum and then back to near zero over a period of about 11 yr. The size of the maximum has varied in the past with a somewhat irregular periodicity of 80–90 yr and there are some grounds for thinking that there is a periodicity of about 180 yr (and perhaps an even longer one). Whether the heat output from the Sun varies with the amount of sunspot activity has not yet been determined definitively. Direct measurements of the solar constant from satellites are not yet adequate to give the answer. Attempts to obtain it from measurements within the atmosphere have likewise failed because corrections for atmospheric turbidity are as large or larger than the changes being looked for and cannot be made with sufficient accuracy.

The views that are held on this matter arise from attempts to relate hemispheric temperature directly to the sunspot number. There seems to be a positive relationship whereby temperature is higher when the sunspot activity is high, but it is a weak relationship with such a low statistical significance that it could



Thermopile for measuring solar radiation (Crown Copyright, Meteorological Office, Bracknell, UK) be a coincidence. As the reliable data on hemisphere temperature extend over little more than one cycle of the 80–90 yr periodicity, this contributes to the uncertainty about the result. There are, moreover, differences in the apparent relationship from epoch to epoch and this is especially notable when we search for the effect in the 300-yr-long temperature record for central England assembled by Manley.

Those who think such analyses nevertheless give a dependable result, that is, higher hemispheric temperatures at the peak of the 80–90 yr cycle, have drawn further support from the analysis of an ice core taken from the Greenland Ice Cap at Camp Century in 1966. This showed prominent cycles at 77 and 177 yr which have been interpreted as being forced by the sunspot variations. The value of this as independent evidence is weakened by the difficulty of constructing the depth/time scale for the ice core and the admission that in this construction an effort was made to preserve natural climatic fluctuations of these lengths, that is, by assuming what we are trying to prove.

The sunspot activity in fact increased with each cycle from 1900, reached a high value in the cycle 1954–64 and fell considerably in the next cycle. It seems a reasonable extrapolation of past solar behaviour to expect the remaining two cycles of this century to show further reductions, and on the basis of the above relationship a number of forecasts of a cooling trend have been made. The proponents of these views rarely state what amplitude the Sun-linked cycles may have. The optimum fit of central England temperature data for spring, summer and autumn—the seasons with the best correlation between temperature and sunspot numbers—for a cycle of 88 yr (that is, eight 11-yr sunspot cycles) gives an amplitude of 0.13 °C which is barely above the noise for this data. Accepting this and placing the maximum in the 1954–64 cycle we should expect the coolest 11-yr period (cycle 1997–2007) to have a temperature 0.26 °C below that of 1954–64. This is for central England data which has in the past displayed variations with nearly twice the amplitude of the Northern Hemisphere data. The amount of depression of hemisphere temperature at the minimum of this cycle around AD 2000 would seem to be at most 0.15 °C. This is just about the amount of cooling between the decades 1955–64 and 1965–74, and would not in any case justify an unambiguous forecast in category (2), that is, a continuance of the cooling trend.

What do we know of the effect of changes in the large-scale circulation on the cloud cover or the distribution of heat between ocean and atmosphere? At present we neither have the knowledge nor the data to know precisely what these changes have been in the past. The intensity of the circulation seems to have been significantly higher during the warming period (1900–40) than in the subsequent years of decreasing temperature. The association of the warming of the Russian Arctic with the increased circulation on the North Atlantic also points to a positive association of circulation strength with hemisphere temperature. The correlation coefficient of 5-yr means of the surface westerlies of middle latitude and hemisphere temperature is, however, not above the chance level. The low correlation arises because the former peaked in the decade 1920–29 and the latter at least 20 yr later. The correlation with the temperature lagging 20 yr is statistically significant but it is not easy to find a satisfactory physical explanation for a lag of this amount. Even if we accept that there is a real association between circulation strength and temperature we need to be able to predict the former to exploit it in a temperature forecast. There is no adequate explanation of the high level of the circulation in the period 1900–40. Some Russian climatologists, among them Vitels (see Lamb[2]), have claimed that the strength of the mid-latitude westerlies is positively related to the long-period sunspot activity. The correlations, however, fall in the range where they could be coincidence especially as the circulation data cover only one cycle.

In an attempt to overcome this difficulty Lamb[2] has assembled data on the frequency of surface south-westerly winds at London going back to 1340 and, assuming these to parallel the strength of the general circulation, has proposed that there is a 200 yr cycle in its strength. On this basis the decline in circulation since 1940 may be expected to continue to the end of this century and beyond. Supposing this to be correct a positive relationship with temperature would then indicate a continued cooling.

Accepting for the moment that the series of south-westerly wind frequencies is homogeneous over the three centuries, there is still the need to show that their frequency is a good measure of the strength of the general circulation. Lamb[2] has not established this beyond a doubt but there looks to be a strong likelihood that this is the case. The next step, that is, establishing a 200-yr cycle, is done less satisfactorily. The argument from the surface wind data seems to rest on the occurrence of times of rapid decline in frequency at intervals of 200 yr from the middle of the sixteenth century to about 1960: this would perhaps establish a saw-toothed highly asymmetrically profile but would seem to leave an uncertainty about further detail in the bulk of each 200-yr period. He adduces evidence of a 200-yr oscillation in solar output as a forcing factor for this putative cycle in the circulation intensity. This evidence comes from variations in ^{14}C concentration thought to be due to variations in the corpuscular and far ultraviolet part of the solar output, the effect of which on the tropospheric circulation has not been satisfactorily established.

Summary of situation

The man-made changes in CO_2 concentration and dust seem to offer the clearest indication of the future trend of temperature. However, the need to explain a cooling over the past three decades in the face of an expected warming due to the additional CO_2 has tended to lead to an exaggeration of the cooling effect of volcanic dust and perhaps even more that of man-made dust. There is also a considerable doubt about how much extra optically effective dust there is on a global basis.

Inasmuch as there is still some doubt about the cause of the bulk of the warming after 1920, a considerable element of uncertainty is introduced into prediction. If the warming was a complicated end-effect of the enhanced circulation during 1900–40 resulting in a reduction in the area of Arctic pack ice and a consequently higher equilibrium temperature due to a reduced albedo, then the post-1940 cooling may be an effect of the reduced circulation. Up to the 1970s, however, it has not been accompanied by a return of Arctic ice to the higher values prevailing at the turn of the century, so that the equilibrium temperature arising from the ice-albedo contribution is probably still well above that around 1900. Does this mean that the full effect of the reduced circulation has not yet been reached? In this connection the failure to explain the lag of temperature behind the circulation is yet another example of how failure to explain the past temperature changes renders prediction hazardous.

In the absence of an explanation based on a consistent theory of climate, prediction would only be reliable if there were some strong and dominant periodic effects. Despite years of search for these and many confident claims of success, none of them stands up well to critical scrutiny. In these circumstances therefore only a great external pressure to make a categorical forecast or an undue attachment to one set of data and its interpretation would seem to explain the abandonment of an agnostic position, or at most the expression of a very cautious probability forecast.

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articles

Vertical particulate spires or walls within the Florida Current and near the Antilles Current

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Spires or humps of particulate material extending from the bottom of the oceanic mixed layer to near the ocean's surface have been observed in the Florida Current and in the regions of other current systems, using an ultrasensitive, 20-kHz acoustical echo sounding system. These spires may be the result of oceanic circulation rather than a purely biological phenomenon.

ACOUSTIC observations of spires of particulate matter extending from the bottom of the mixed layer to near the surface of the ocean have been made in the Florida Current (November 1977), in the region of the Antilles Current (January 1973) and in the Caribbean (April 1978). The Florida Current observations were made in the vicinity of 80° W long, and in latitudes between 25 and 28° N; Antilles Current region observations were made in around 70° W long, 25° N lat, and the Caribbean observations were made in a region ~200 nm north of Caracas, Venezuela. The observations were made using an ultrasensitive, short pulse length (<1 ms), towed, narrow beam (18°), 20 kHz acoustic echo sounding system. The use of this system to detect layers of particulate matter and internal waves in the ocean has previously been documented[1,2]. The acoustical frequency (20 kHz) and receiver sensitivity are such that scattering from oceanic turbulence or density gradients is not detectable, so that particulates and/or biota are the most likely sound scatterers detected.

Acoustic and XBT data

Figure 1 shows real-time acoustic data obtained from the RV Researcher while crossing the Gulf Stream off Palm Beach, Florida. The ship proceeded due east from Palm Beach, at a speed of 2 m s^{-1} beginning in water of depth 220 m. A layer of particulate matter corresponding to the depth of the bottom of the mixed layer may be seen gradually descending spatially from left to right (labelled A in Fig. 1). Lines of constant density (isopycnals) are known to slope down proceeding from west to east across the Gulf Stream[3]. The slope of the reflecting layer has a value $\sim 4\times10^{-3}$ which compares favourably with the 3×10^{-3} isopycnal slope, a value deducible from the isopycnal data of Schmitz and Richardson[3]. Interference lines generated by the Researcher's echo sounder are indicated and should be disregarded.

The most interesting features are the spires of particulate material which are seen to extend vertically from the particulate layer corresponding to the bottom of the mixed layer. Figure 1 shows that the spires eventually disappear in a shorewards direction; the mean horizontal spacing of the spires is ~200 m. Spire widths at the base of a spire seem to be 100 m or more. An important characteristic of the acoustical system used in gathering the data presented in Fig. 1, is that it was not designed for obtaining data near the ocean's surface, say, within 10 m. Accordingly, information or acoustical signals coming from this region is suppressed by the electronics. Thus it is uncertain whether the particulate spires observed in Fig. 1 reach the ocean surface.

Expendable bathythermograph (XBT) records were gathered at the same time as for the acoustic data. Three XBT records taken at 21.30, 21.45, and 22.00 LT, are shown in Fig. 2. Note that the temperature profiles are essentially featureless down to the depth of the acoustic reflecting layer, at which depth small temperature gradients are visible. Previous experience[4-6] has shown that a particulate layer is often observed in coincidence with a temperature gradient. In addition to the shallowest temperature gradient (clearly visible at 45 m), the temperature profile gathered at 21.45 LT has two small temperature gradients (I and II in Fig. 2; with the corresponding reflecting layers marked I and II in Fig. 1).

From the data of Schmitz and Richardson[3], it is reasonable to expect that the northwards surface current varies from ~100 cm s^{-1} at the left of Fig. 1, to ~180 cm s^{-1} at the right of

Fig. 1 Acoustic reflecting layers and particulate columns observed while crossing the Gulf Stream: interference lines are labelled (i).

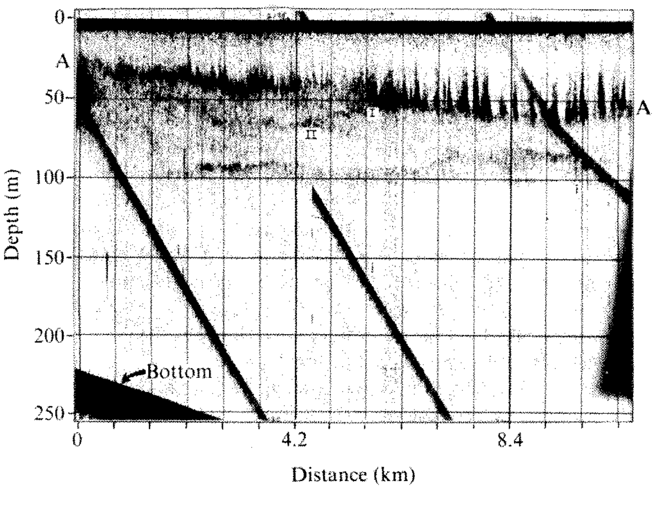

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Fig. 1. The mean eastwards current is very small, being maximum at the right of Fig. 1, with a magnitude no larger than $\sim 5\ \text{cm s}^{-1}$.

The acoustical data shown in Fig. 1 are greatly exaggerated in the vertical; Fig. 3 is a geometrically corrected interpretation of the data between the 7.7 and 8.8 km range limits of Fig. 1. Figure 3 shows that what we are calling spires, because of their appearance of the acoustic records, are in fact more like humps or mounds. 'Hump' may not be an adequate description, however, because the along-stream extent of a particular hump may greatly exceed its cross-stream extent; in which case perhaps the term 'wall' would be more appropriate. There is acoustical evidence that the along-stream length of a hump or spire could be >1 km.

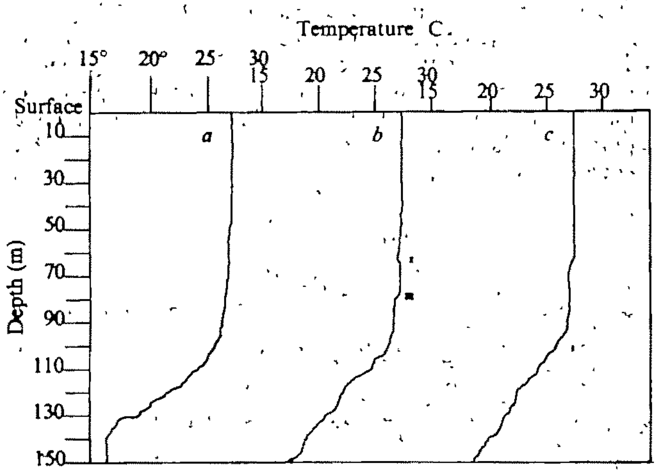


Fig. 2 Temperature profiles corresponding to the acoustic data in Fig. 1. *a*, T-11-38, 21.25LT; *b*, T-11-39, 21.43LT; *c*, T-11-40, 22.05LT.

Vertical structures

We can be certain that the vertical structures were observed in each cross-stream traverse made by the ship between longitude 28 and 25° N, but whether given spires in a crossing along-stream are continuations of given spires in another traverse is not.

The columns shown in Fig. 1 have a vertical extent, L, which is of the order of the depth of the bottom of the mixed layer, that is, if d is the depth of the bottom of the mixed layer, then

$$L \sim d \tag{1}$$

The spires have a horizontal spacing, h, which on average seems to be ~2–4 times the depth of the mixed layer:

$$2d \leq h \leq 4d \tag{2}$$

This conclusion is reached by examining substantial additional data to that shown in Fig. 1. Not all the spires are of equal size and dimensions; equation (2) is an average statement and implies that as the depth of the thermocline changes, as occurs in traversing the Gulf Stream, so too should N (the number of spires per unit horizontal distance) vary:

$$\frac{1}{4d} \geq N \geq \frac{1}{2d} \tag{3}$$

The spire width, w, at the spire base in the cross-stream direction is of the order of 2–5 times the mixed layer depth:

$$2d \leq w \leq 5d \tag{4}$$

A typical dimension for d is 50 m, so that $L = 50$ m, $100\ \text{m} \leq h \leq 200\ \text{m}$, $0.005\ \text{m}^{-1} \leq N \leq 0.01\ \text{m}^{-1}$, and $100\ \text{m} \leq w \leq 250\ \text{m}$. The spire structures persist over a 24-h period and are not changed due to the rise of the deep scattering layer (DSL). In fact, the DSL rises to the thermocline depth and then contributes (for example, biota) to the particulate spires.

Table 1 Data from observations of vertical particulate spires

Date	Location	Time interval of observation	Wind speed (m s^{-1})	Wind direction
19 Mar. 1978	14°12′ N 66°39.0′ W	20.56–22.06 LT	10	090°
11 Nov. 1977	28°59.5′ N 79°07.1′ W	08.30–13.00 LT	7–9	350–000°
11 Nov. 1977	29°03.9′ N 80°04.3′ W	19.00 LT–01.00 12 Nov.	8–10	330–350°
12 Nov. 1977	29°14.1′ N 79°42.7′ W	18.00 LT–06.30 13 Nov.	10–12	330–360°
14 Nov. 1977	26°49.3′ N 79°54.1′ W	21.00–23.30 LT	7	065–085°
31 Jan. 1973	25°21.8′ N 71°12.2′ W	11.35 LT	10	080°

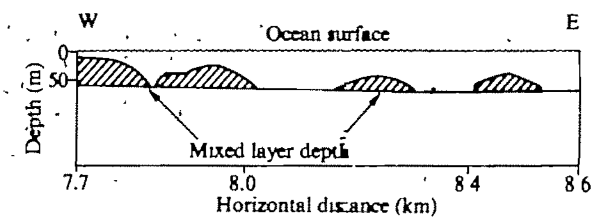
As we have stated, we are uncertain about the phenomena generating and sustaining the particulate spires. However, the hypothesis for the origin of the spires (or, perhaps walls) has two parts. First, the spires are not primarily the result of biological behaviour. The particulates comprising the reflecting layer, out of which the spires extend, are thought to be plankton, and in the case of the Florida Current, at least, are also thought to be sediment, perhaps in combination with biota. It is unlikely that such particulates are highly mobile, and, in any case, there is no report of innate particulate spire-forming behaviour for oceanic marine biota. Second, the spires arise from oceanic water circulation and congregate in spires (or walls) as a result of that circulation.

Water circulation

If the particulate structures arise from water circulation, what would be the form of that circulation? The structures might be formed by an upwards directed flow so that the particulates are carried upwards from the reflecting layer. If there is such a vertically directed flow, then only the uppermost reflecting layer contributes particulates to the flow. This can be seen by examining some of the deeper reflecting layers in Figs 1 and 4. No particulate spires extend from any other than the shallowest reflecting layer which suggests that the vertical flow is confined between the thermocline and the surface.

We feel that the proposed circulation is not due to the presence of internal waves. As may be seen from Figs 1 or 3, little or no vertical spatial variation of the shallowest reflecting layer appears on the 50–500 m length scale. Internal waves of this horizontal scale, of amplitude as small as a couple of metres, are detectable with this acoustic system[2]. However, small scale perturbations are visible on some of the deeper reflecting layers in Fig. 4. The kind of wind conditions at the time the spire observations were made may be relevant to whether circulation cells of some type are present. For example, Faller[7] has shown that certain wind and wave conditions must be present for Langmuir type circulations to occur. Table 1 shows wind and other data for various spire observations: such as measured winds exceed $5\ \text{m s}^{-1}$ in all observations. This contradicts the

Fig. 3 Interpretation of the acoustic data in Fig. 1 from 7.7 to 8 km with the vertical exaggeration removed.



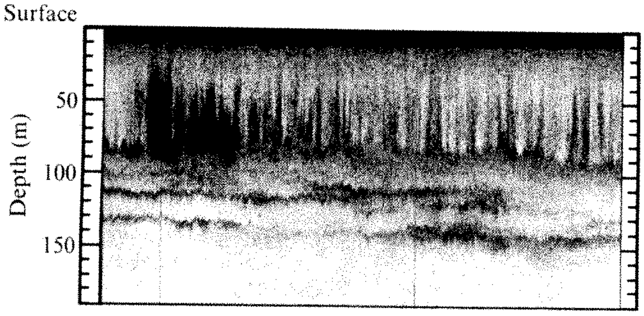


Fig. 4 Acoustic reflecting layers and spires in the area of the Antilles Current.

results reported in ref. 8: in which it is stated that the "columnar scattering" is observed in the wind mixed layer during very calm weather conditions. Table 1 also indicates that observations of spires were made during daylight and night-time conditions. We do not wish to speculate any further, questions as to whether the flows are some sort of circulation cells (so-called Langmuir cells) must be put off for the moment.

Implications of particulate spires in the Gulf Stream

One of the most important implications of the existence of particulate spires in the Gulf Stream is the small scale variability in the horizontal of particulate densities. Figure 1 shows the small scale variability of particulate densities, both in the vertical and the horizontal. While on-station, the particulate columns have the appearance of patches as the ship slowly drifts. Depending on the along-stream length of the cells, there may be a distinct difference in a biological net tow, depending on whether the tow is along-stream or cross-stream. The behaviour in the deep scattering layer, mentioned above, is interesting in that the DSL is generally thought to be mobile, but, nevertheless, preferentially aligned above the thermocline into the spires.

There are also some interesting potential implications for ocean-mixed layer studies; particularly if spire formation indicates an underlying circulation. As Pollard[9] pointed out, circulations such as those of Langmuir are potentially efficient in mixed layer momentum redistribution. A major question remains: how widespread or typical are the phenomena described here? We do not know, for example, if such spires are in some way related to the presence of ocean currents. The data gathered in Fig. 1 were clearly in a current system—the Gulf Stream. The observations shown in Fig. 4 may have been of a less well known current system[10].

Conclusion

Spires of particulate matter have been observed extending from the bottom of the mixed layer to near the surface of the ocean and in the region of different ocean current systems. The detection of the spires was made possible through the use of an ultrasensitive 20 kHz acoustical system. Further research is required into the nature of the particulates present and of the phenomena generating the spires.

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Protein disk of tobacco mosaic virus at 2.8 Å resolution showing the interactions within and between subunits

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Protein subunits in the two layers of the disk of tobacco mosaic virus have very similar conformations. Much of the bonding between subunits is polar, including salt-bridge systems. Arginine residues play a prominent part here and elsewhere. Interactions within each layer involve groups whose contacts can be adjusted to allow the transition from disk to virus helix. Aromatic clusters within each molecule are linked in a hydrophobic girdle encircling each ring.

THE disk of tobacco mosaic virus (TMV) protein (see review by Caspar[1]), is an obligatory intermediate in the assembly of the virus from its constituent RNA and protein[2]. It fulfils both the physical requirement for nucleating particle growth and the biological requirement for specific recognition of the viral RNA. The disk is composed of two rings or layers, each containing 17 subunits[3] and both facing the same way[4]. The two layers are in contact only at high radius whereas at the opposite end of a subunit, towards the central hole of the disk, they are separated as if poised for reaction with, and intercalation of, the viral RNA[5]. Assembly is initiated by insertion into the middle of the disk[6] of a special sequence within the RNA[7] which folds into a hairpin loop[8]. Interaction with this loop has several consequences for the protein subunits: the circular subunit arrangement of the disk becomes the helical one of the virus; the two rings close about the bound RNA, losing the pairing interaction between them at high radius; the flexible part of the protein at low radius assumes the ordered structure of the virus[9]. During these rearrangements all the lateral contacts between subunits are essentially preserved while the axial ones are completely changed.

We have previously described[5], at 5 Å resolution, the subunit packing within the disk and the folding of the polypeptide chain. Extension of the analysis to 2.8 Å resolution provides details, as described here, of the conformation of the subunits and the variety of their interactions. Subunits in the two layers are remarkably similar despite their crystallographic independence. Notable features of the structure include the important structural roles of several arginine residues, the extended salt-bridge

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Table 1 Statistics of data collection

	Native	Mercury
Total reflections measured	817,000	626,000
No. of independent reflections	200,000	163,000
$R_{\mathrm{sym}} = \Sigma_i\lvert\bar{F}-F_i\rvert/\Sigma_i F$	0.10	0.11
R_{sym}, 50% strongest reflections	0.08	0.09
Mean isomorphous difference $\langle\lvert F_{\mathrm{nat}}-F_{\mathrm{der}}\rvert\rangle/\langle F_{\mathrm{der}}\rangle$	—	0.30

Using the methods of oscillation photography[13], with a range of 0.5–0.75° for a single picture, a total of about 230 film packs were used to cover the complete 90° oscillation range about the *b* axis, and the 32° of cusp data about the *a* axis, which together constitute a complete set of data. A typical film pack required an X-ray exposure of about 10 h. The crystal lifetimes varied widely up to a maximum of about 50 h exposure for any one location, with translation of the crystal giving up to three locations per crystal. Overlapping rotation ranges were used whenever the crystal location was changed. The fully and partially recorded intensities were measured with a flat-bed hybrid densitometer[14]. Films were scaled together by means of symmetry-related reflections recorded on different films and a symmetry *R* factor was computed for each compiled set of data. Reflections split between two contiguous rotation ranges were not used for scaling but are included in the statistics They comprise 40% of the total measured reflections and are not quite as accurate as comparable fully recorded reflections, but the compiled sets of data would have been far less complete without them.

networks both within and between the rings, and the encircling of each ring by a complete girdle of hydrophobic residues.

Structure analysis

Preparation and crystallisation of the disk protein have been described previously[10]. The crystals are orthorhombic, space group $P22_12_1$, with unit cell dimensions $a = 228$ Å, $b = 224$ Å, $c = 174$ Å. There is one disk of molecular weight 600,000 per asymmetric unit[11] with its 17-fold axis approximately parallel to *c* (ref. 12).

X-ray intensities were recorded by an extension of the methods of oscillation photography[13] used for the 5 Å data and described elsewhere[5]. Almost 2×10^6 intensities were measured[14] for crystals of the native protein and a single covalently bound mercury derivative[10] (Table 1). The derivative crystals did not diffract as well at higher resolution as those of the native protein, and thus the mercury data effectively extend only to the slightly lower resolution of 2.95 Å. This was compensated for by use of the 17-fold non-crystallographic symmetry[11,12] to extend, as well as improve, the phases from the single-isomorphous derivative. Heavy-atom parameters were refined independently for the 34 mercury atoms in a manner similar to that used at 5 Å resolution[5]. Details of these calculations will be published elsewhere. Phase refinement by non-crystallographic symmetry averaging[15–17] proceeded in two stages (Table 2) to investigate the existence of any departures from the 17-fold symmetry. However, no such departures were found. The averaged map resulting from stage I of this phase refinement (cycle 2 map, 1976) was used in an optical comparator[18] to build an atomic model of the protein subunit which was verified by the cycle 5 map (1977) from stage II of the refinement. This map was used both for making minor adjustments to the physical model and for model-building with a computer-graphics program (BILDER)[19]. All the atomic coordinates used in the figures accompanying this paper are taken from this computer model. Parts of the model are shown in Fig. 1 superimposed on the cycle 5 electron-density map.

Description of the subunit

The molecular boundary, the course of the polypeptide chain and the strong similarity between subunits in the two rings, which were clear at 5 Å resolution, are all confirmed at higher resolution. The chain-tracing shown in Fig. 2 differs from that based on the 5 Å map only in that the wrong alternative was then chosen for the connectivity of the four-way contact at 70 Å radial distance from the centre of the disk. In no way does this affect the sequence of the four main helices, the only consequence being to reverse the direction of the chain through the 'hanging loop' from that proposed earlier.

Detailed interpretation of the map has led to an atomic model of a subunit in the B ring of the disk and all its contacts with neighbouring molecules. The densities for a subunit in the two rings of the disk are so similar that any differences in molecular conformation between the rings will be assessable only after refinement of the molecular coordinates, which is now in progress, has been completed. Examination with a computer-graphics program[19] of the model for a subunit in ring B superimposed, by a rigid-body transformation, on the density for a ring A subunit showed that the atoms in the main chain were all within 0.5 Å of the positions indicated by the density and that most of the side chains were equally close.

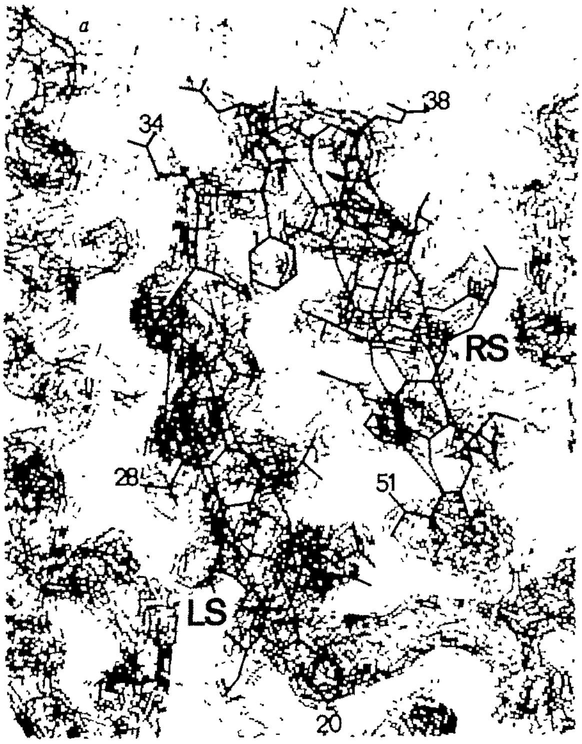
Table 2 Progress of the phase determination

	Stage I			Stage II			
Cycle	0	1	2	2*	3	4	5
*R factor after averaging		0.47	0.29	0.41	0.37	0.31	0.28
Correlation (between F_{obs} and F_{calc})		0.63	0.85	0.69	0.75	0.82	0.86
Figure of merit	0.48	0.63	0.78	—	—	—	—
Mean phase change from last cycle		41°	11°	41°	37°	13°	7°

*$R = \langle\lvert F_{\mathrm{obs}}-F_{\mathrm{calc}}\rvert\rangle/\langle F_{\mathrm{obs}}\rangle$. Where F_{calc} is the structure factor computed from the averaged map.

The initial data set consisted of 145,000 amplitudes phased by single isomorphous replacement (SIR) with a mean figure of merit of 0.48, and of 55,000 unphased amplitudes, mostly between 3 2 and 2.8 Å resolution. Heavy-atom parameters had been refined independently for the 34 mercury sites. The parameters of the 17-fold non-crystallographic symmetry axis were then checked but found to be unchanged from those determined at 5 Å resolution. The phase refinement was done in two stages, to settle any doubt as to whether the absence of density at low radius in the 5 Å map was due to disorder or to an abuse of 17-fold averaging. For stage I the electron density was averaged only in that region of the disk lying between 40 and 90 Å radius, and was left unchanged in the rest of the asymmetric unit. This considerably weakened the phase constraints imposed by the symmetry, as the *U*/*V* ratio[16] was 0.6, but combination of phase information from SIR and from this partial averaging still acted as a constraint, and produced a marked improvement in the overall quality of the map. However, no significant departures from 17-fold symmetry could be detected, either at low radius or at contacts between adjacent disks. These unconstrained regions were rather noisy, and this noise tended to correlate with itself from one cycle to the next, leading to spuriously low residual *R* factors. For stage II, the density was 17-fold averaged in the whole disk at all radii above 20 Å, and was reset to its average value outside the disk boundaries. Deep holes of negative density at the heavy-atom sites, due to poor SIR parameters, were removed from the maps. The phases calculated from such maps were associated directly with the observed amplitudes, without weighting and without recombination with the isomorphous phases, to produce the structure factors used in the next cycle. The use of this new procedure, starting from the phases of cycle 2, induced a large readjustment of the poorer phases (cycle 2*), followed by convergence. The constraints imposed by the 17-fold symmetry were 13.5 times as strong ($U/V = 0.045$) as in stage I, yet a comparably low residual factor was obtained. This strongly suggests that all the ordered density in the disk accurately obeys the 17-fold symmetry.

The central part of the molecule consists of a group of four α helices which are arranged closely parallel or anti-parallel to each other and extend between 45 and 65 Å radius[5]. The left and right slewed helices, LS and RS, in the upper half of the subunit are joined at the end proximal to the disk axis by a short bend in the protein backbone referred to as the slewed hairpin. The right and left radial helices, RR and LR, in the lower half of the subunit both fade into weak discontinuous density, indicative of the flexibility of this loop region of the protein in the



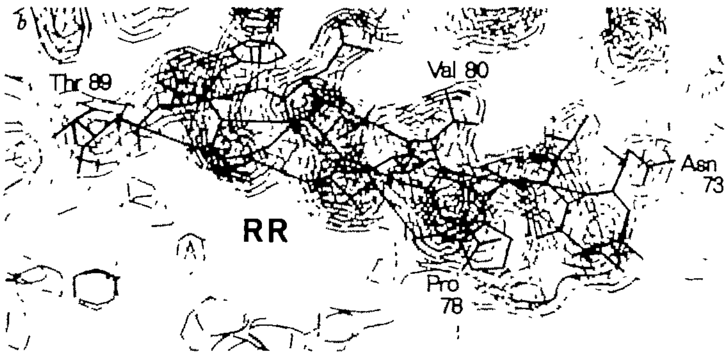


Fig. 1 The electron-density map (cycle 5) for three of the helical regions of the structure with the model superimposed. For continuity, the atomic skeleton is shown over a greater depth than the map. This is contoured at equal intervals of 0.12 e Å^{-3} above zero with the F(000) term excluded. *a*, A slab 6.5 Å thick through the two slewed helices and their connecting hairpin loop, viewed from above. The left slewed helix LS begins at Pro 20 and terminates with a bifurcated hydrogen bond from the peptide NH of residue 33 to the carbonyl groups of residues 29 and 30 which are in the α- and 3_{10}-helical positions, respectively. The right slewed helix RS terminates in similar fashion, with the carbonyl groups of residues 47 and 48 sharing the hydrogen from the peptide nitrogen of residue 51. The RS helix begins with a hydrogen bond from the hydroxyl of Thr 37 to the peptide NH of residue 40. The loop connecting the helices includes Glu 34 which forms an inter-subunit hydrogen bond with Thr 42, Phe 35 which points back into the hairpin between the two helices, and three invariant residues Glu 36, Thr 37 and Glu 38 which all point inwards towards low radius where they are exposed to the solvent. *b*, A slab 5 Å thick through the right radial helix RR, viewed from the side, showing Pro 78 contained within this helix which starts at residue 74 with a bifurcated hydrogen bond from the peptide carbonyl of 73. The proline prevents the formation of two potential hydrogen bonds within the helix, those which would have involved the NH groups of residues 78 and 79, and causes the helix axis to become more closely horizontal at higher radius. The two peptides whose carbonyl groups are unable to form hydrogen bonds are tilted away from the regular helical conformation, exposing them to the solvent. The helix continues to Asp 88 where the density then becomes very faint, with Thr 89 probably not in a helical conformation but only weakly indicated in this map.

absence of RNA[5]. The flexible loop which connects these radial helices at their proximal ends is, in the virus, located at lower radius than any other part of the protein[9]. The distal ends of all four helices are braced together by a short anti-parallel β sheet of four strands. Amino acid side chains on the high-radius side of this sheet form part of a hydrophobic girdle which extends continuously around the circumference from one subunit to the next. The girdle also includes residues from the upper loop, a piece of extended chain between residues 54 and 67, joining the RS and RR helices, the hanging loop extending from the N terminus towards residue 16 of the β sheet and the RS helix, and the C-terminus region arising from the β bend at the end of the LR helix. Within this girdle is an extended aromatic cluster in the centre of each subunit and a smaller such cluster across the subunit interface. Elsewhere in this interface and that between the two layers, there are systems of salt bridges involving some of the many arginine residues present in TMV protein. Arginine residues play an important part in both intra- and inter-subunit interactions, exploiting fully the high specificity and high directionality of the bonds which can be formed. These various elements of the structure will be considered in turn.

The α-helical core and the β sheet

The four main α helices include about 60 residues out of the total of 158 (ref. 20), with residues 20–32, 38–48, 74–88 and 114–134 occurring in the LS, RS, RR and LR helices, respectively. Residues 49–52 form a 3_{10}-helical extension of RS at its distal end. All the helices are somewhat distorted from the idealised structures. The helices are packed as in a segment of a left-handed, 4-stranded supercoiled bundle with hydrophobic contacts along the centre. This hydrophobic core contrasts with the many polar and charged groups on the outside of the supercoil where there are alternating patches of polar and hydrophobic residues. The ends of the helices are orthodox, with bifurcated hydrogen bonds finishing off both LS and RS (Fig. 1*a*) and starting off RR (Fig. 1*b*), a hydrogen bond from a side chain at the beginning of RS and a β bend at the end of LR. Whereas Pro 20 starts the LS helix in the usual manner, the position of Pro 78 within the RR helix is highly unusual. The proline distorts the helix, causing its axis to incline more steeply upwards towards lower radius, thereby preventing the formation of two of the helical hydrogen bonds (Fig. 1*b*).

The hairpin bend joining the slewed helices contains the highly conserved group of residues 33–38. Phe 35 extends back inside the hairpin with its aromatic ring between the pair of helices in a similar manner to the packing observed in myoglobin of Phe 123 between the G and H helices[21]. Invariant glutamines 36 and 38 extend inwards towards low radius, suggesting an availability for interaction with nucleic acid. Other residues in this loop form bonds to the lower half of the subunit. The flexible loop of residues 89–113 connecting the radial helices is ordered only in the presence of RNA and so the map has no interpretable density at 20–40 Å radius, where these residues would be expected, and only weak density for residues 114–116 in the LR helix. When the mobility of the loop[22] is reduced by nucleotide binding to the disk, it seems, as discussed below, that the LR helix begins properly at residue 109 or 110. The RR helix terminates abruptly after Asp 88, which forms an inter-subunit salt bridge with Arg 122.

The distal ends of the four helices are connected transversely by a narrow and twisted strip of β sheet shown schematically in Fig. 3. The four main strands of the sheet tie the helices together in pairwise fashion with the two slewed helices above linked to the upper two strands of the sheet and the two radial helices below to the lower strands. The two central strands of the β strip are themselves connected through the high-radius upper loop. This loop and the other connecting loops in the sequence do have some very short helical segments (α and 3_{10}) but few of these are long enough to have even a single helical hydrogen bond.

The hydrophobic girdle

The central part of each subunit on the distal side of the β sheet is a cluster of aromatic residues: Trp 17, Tyr 70 and Tyr 139 from the sheet, Phe 12 and Phe 144 from the two terminal loops, and Phe 62 from the upper loop. These groups, together with Pro 56 and Pro 63, form an extended cluster in which the rings are mostly in an edge-to-face arrangement. At the same radius of 75 Å, but extending across the molecular interface, there is a further cluster composed of Pro 20 and Phe 67 with Pro 7 and Phe 10 of the neighbouring molecule. These two clusters are joined by several hydrophobic aliphatic side chains, arising from elements of all four chains which cross this radius, to give a continuous belt of hydrophobic interactions encircling each ring of the disk.

Salt-bridge systems

The subunits in the two layers have three regions of interaction (Fig. 4) of which the largest is the extended salt-bridge system shown in Fig. 5. This involves the only two lysine residues and four acidic groups, from three segments of the main chain in the lower ring with Asn 127, Glu 131 and Arg 134 from the LR helix in the upper ring. Almost all the possible hydrogen-bonding capabilities are utilised in this complex 3-dimensional network, in which two water molecules also participate. Note that the disk is stable in very high ionic strengths[23,24], so that a simple view of the instability, in high ionic strengths, of isolated salt bridges does not apply to an extended system like this with hindered access to solvent.

Adjacent subunits within a ring are linked at low radius by a simpler salt-bridge system involving Arg 122 and Asp 88. In this case also, most of the hydrogen-bonding potential of the arginine is utilised, with additional links to Thr 89 in the neighbouring subunit and to the peptide carbonyl groups of residues 31 and 33 in the same subunit.

At higher radius in the same molecular interface (Fig. 2), the region of polar interaction has an intra-subunit salt link involving Arg 71 and Asp 77 with additional hydrogen bonds to Thr 81, Ser 49, and an inter-subunit link to Thr 28. In the cowpea strain of the virus, however, coordinated sequence changes[25] result in this region also having an inter-subunit salt linkage.

Role of arginine residues

The basic residues in TMV comprise 2 lysine, 11 arginine and no histidine residues[20]. The role of the lysine residues and three arginine residues (134, 122 and 71) in various salt-bridge systems of interaction within the subunit interfaces has been discussed above. In the subunit itself there are also two simple salt linkages involving Arg 141 with Asp 64 and the invariant pair Arg 61 with Glu 145. Both these pairs are exposed on the outer circumference of the disk where they link the C-terminal and upper loops of the protein.

Interaction with RNA is expected to involve three arginine residues[1] out of the four invariants (41, 90, 92, 113) which occur at low radius. Arg 41, the only such residue seen in our map, seems less likely to have such an interaction than the other three whose positions, as extrapolated by model-building from positions 89 and 114, would have them all pointing down from the A ring into the 'jaws' between the layers of the disk.

The high specificity and directionality of the polar bonds formed by arginine residues contrast with the hydrophobic surfaces presented by their methylene chains. That of Arg 61 lies against the planar face of Trp 152, and similarly Arg 122 is close to Leu 31, and Arg 41 lies between the rings of Phe 35 and Phe 87.

Inter-subunit contacts

Contacts between the layers: Three regions of contact are indicated in Fig. 4, all of different type and all occurring at the higher-radius end of the molecule but spaced apart. The outermost is a polar interaction between serines 147 and 148 and Thr 59. At lower radius are the extended salt-bridge system described above and also a small hydrophobic patch where Ala 74 and Val 75 both touch the ring of Pro 54. These three contacts form an essentially kinematic mounting of a subunit of

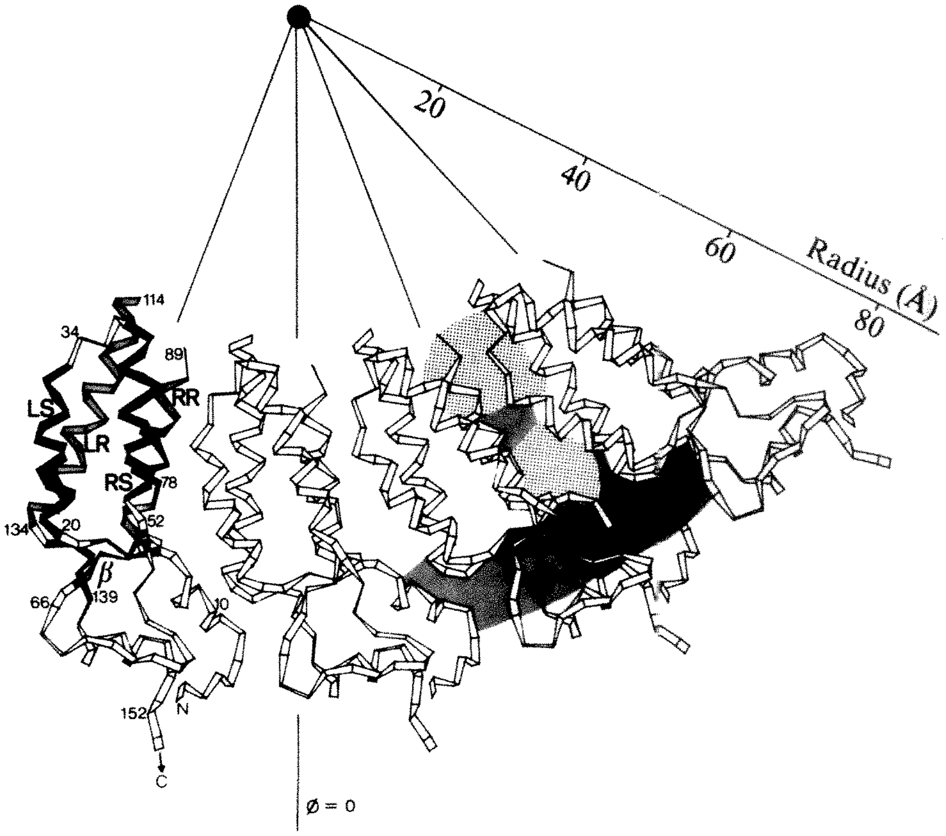


Fig. 2 The folding of the polypeptide chain and the lateral interactions between subunits. Four adjacent subunits within a ring are shown viewed from above. The N and C termini of the chain are marked on the extreme left subunit together with the four main helices, left and right slewed (LS and RS) in the upper half of the molecule with right and left radial (RR and LR) below, and the β sheet which connects all four helices at their higher radius ends. The other subunits show the overall shape of the subunit as a scimitar, with a pronounced slew relative to the radius of the ring. The interface contains alternating patches of polar residues, indicated by stippling, and of hydrophobic residues, indicated by solid shading, which are all contributed to by residues from both the subunits forming the interface. The hydrophobic patch at higher radius is continuous with the hydrophobic girdle which extends circumferentially across the whole width of a subunit and across the interface, forming a continuous belt around the ring. The hydrophobic girdle includes residues from the high radius side of the β sheet, the upper loop which joins the RS and RR helices and the two lower loops leading to the termini of the chain.

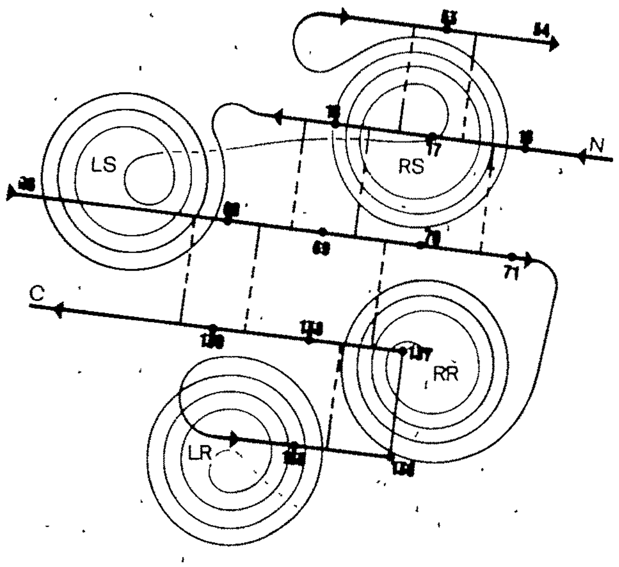


Fig. 3 Schematic view of the anti-parallel β sheet showing the connectivity of the strands and of the four principal α helices. The direction of view is along the long axis of the subunit towards the centre of the disk. The β sheet is a narrow, twisted strip which extends across the subunit bracing together the four α helices. At the bottom of the β sheet is a β bend which occurs immediately after the end of the LR helix. The bend is of type II where the second residue is commonly glycine and indeed Gly 137 is invariant in all known strains of TMV. The residues whose side chains are on the higher-radius side of the sheet are the two lysines (53 and 68), which form part of an extended salt bridge (Fig. 5), and three aromatic residues (Trp 17, Tyr 70 and Tyr 139) which form part of a cluster within the continuous hydrophobic girdle.

one ring onto one of the other. They are completely changed during the transition from disk to helix.

Contacts within a layer: By contrast, the four patches of alternating polar and hydrophobic contacts (Fig. 2) are maintained during the disk to helix rearrangement. There is no evidence of any relative motion within a subunit during this transition, and the coordinates of heavy-atom labels at four equivalent sites[26] in the disk and the virus show that the molecule moves as a rigid body[24]. Considerable flexibility is thus required in the side-chain contacts if the backbone conformation stays essentially rigid. During the transition, subunits in the A and B rings tilt[27] by 10° and 20°, respectively, about a tangential axis[5,9], so changing by 4° and 8° the hinge angle at the interface between neighbouring subunits, if these are envisaged as being related by a hinge extending radially along the interface. This change in hinge angle could mean an increased distance between the slewed helices in the upper part of the interface, a decreased distance between the radial helices in the lower part, or both of these. The first alternative is the least likely, as the transition results in a more compact structure[6] in which the subunits are closer to the central axis and the number of subunits per turn is decreased from 17 in the disk to 16⅓ in the virus. Figure 2 shows that in the disk the interface has more space between the radial than the slewed helices to allow the transition. Both the polar patches in the interface have residues which act as spacers: Arg 122 in the patch proximal to the axis and Tyr 72 in the distal patch. Rearrangement of the packing of these side chains allows the necessary movement in the polar patches. The non-directional character of the interactions in the hydrophobic patches could allow the apposed surfaces there to slide over each other during the transition.

Comparison with virus

In describing the structure of the RNA and its binding site, as deduced from a map of the virus itself using 4 Å data, Stubbs *et al.*[9] presented a model for the four main α helices which differs in several respects from that presented here. The largest differences are in the radial helices. Tyr 139 is located by a label in each model[24] and, whereas our model has a compact β bend separating this from Arg 134, the virus model has a greater separation. Comparison of our map with the 5 Å resolution map of the disk containing bound oligonucleotides[28], and computer model-building[19] indicate that, in the presence of bound nucleotide, the LR helix continues inwards to residue 109 or 110 before the chain turns upwards into the vertical column of density (V) seen in the virus map[9]. In the virus model this turn occurs at position 113, a difference of +3 or +4 residues between our model and the virus model. In our RR helix, Pro 78 occurs right at the bottom of the subunit marking a turn where the helix axis becomes more closely horizontal with increasing radius, whereas in the virus model the chain becomes less horizontal and then continues out in radius to residue 70. In our model we place Tyr 72 at the end of the radial density and residues 71 and 70 lie much higher than this radial helix (Fig. 4). None of the helices in the disk model have the idealised geometry which was built into the virus model, with the result that, although the two

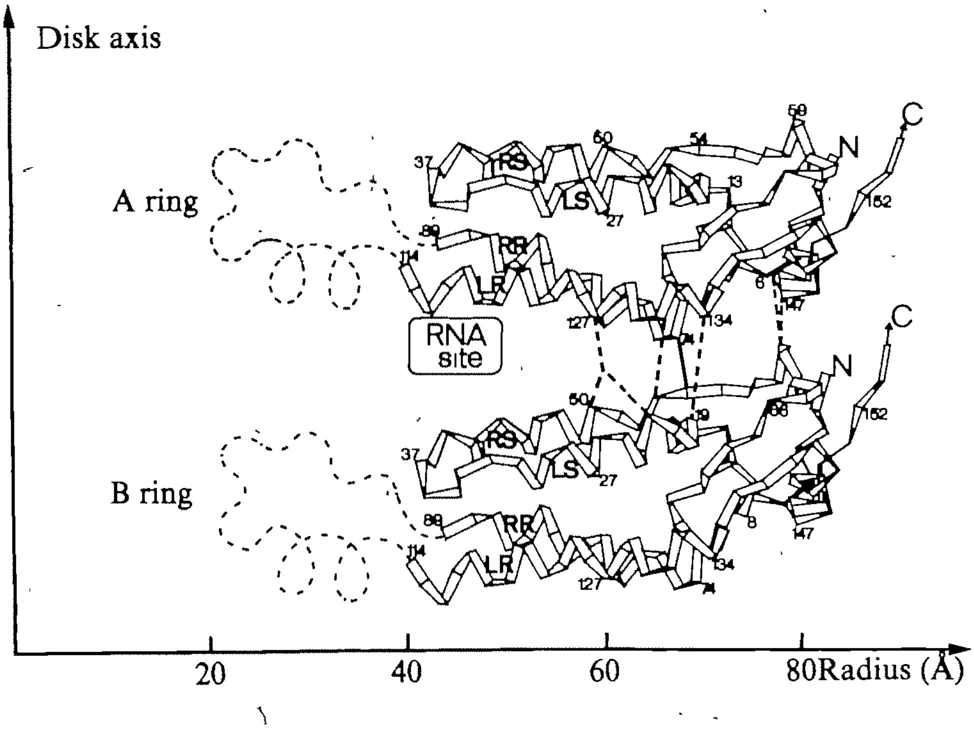


Fig. 4 Side view of a sector through the disk showing the relative disposition of subunits in the two rings and the axial contacts between them. There are three regions of contact indicated by a solid line for the hydrophobic contact of Pro 54 with Ala 74 and Val 75, by dashed lines for the hydrogen bonds between Thr 59 and serines 147 and 148, and further dashed lines for the extended salt-bridge system shown in greater detail in Fig. 5. The low-radius region of the chain, which has no ordered structure in the absence of RNA, is shown schematically with an indication of an additional 1–2 turns extending the LR helix (see text) before it turns upwards into the vertical column. The primary nucleotide-binding site is probably below the RR helix[28].

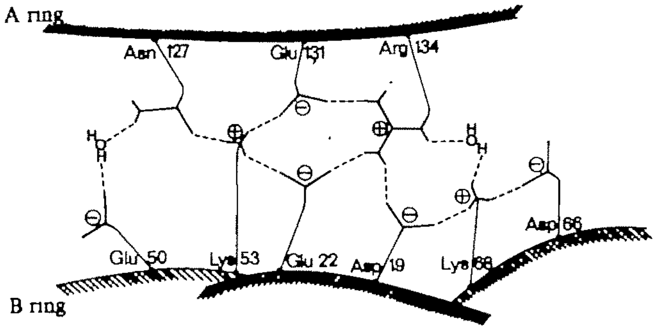


Fig. 5 The extended salt-bridge system between subunits in the two rings of the disk presented schematically in a side view. All three residues in the A ring emanate from the LR helix whereas those in the B ring arise from the 3_{10} extension of the RS helix and the top strand of the β sheet (50 and 53), the beginning of the LS helix (19 and 22) and lastly, the end of the upper loop and the third strand of the β sheet (66 and 68). The salt-bridge system, which utilises almost all possible hydrogen-bonding sites, is a complex three-dimensional network which is only presented here schematically. There are clear features in the electron-density map to represent both water molecules.

polypeptide chains may be out of phase by −2 residues beyond the distal end of the RR helices, both models can still have, for example, Asp 88 pointing out across the subunit interface. In the LS helix Cys 27 is located by a mercury site in both the disk[12] and the virus[29] but, whereas in our model Cys 27 points downwards towards the centre of the four-stranded supercoil, in the virus model it faces the RS helix[9], indicating a difference of +1 residue between the models.

The RR helix in the virus model continues for two or three turns beyond Asp 88 where, in the disk, it terminates at the start of the flexible loop. Extension of the RR helix beyond this point is not possible in our model because of interference from residues 38 and 41. Preliminary evidence from a low resolution study of oligonucleotides bound to the disk[28], while suggesting that the LR, LS and RR helices all move slightly upwards, still gives no clue as to how the flexible loop is folded. However, the different assignments of phase in the α helices mean, first, that our model has fewer residues to be fitted in between this point and the start of the LR helix and thus they need not be as tightly packed as the α helices of the virus model; and second, that the existence of a carboxyl cage[9], so-called in the virus model, must be treated with caution although, purely on the basis of the sequence, there must be a concentration of negative charges in this region of the molecule.

Discussion

The protein of TMV is the only protein from a large macromolecular assembly whose structure is now known at this level of detail. The subunit is a small protein folded into a single domain within which the folding of the amino and carboxyl halves of the polypeptide chain is quite similar. Between these two parts of the molecule there is an approximate twofold symmetry, which is geometrical as well as topological, indicative of a possible gene duplication (A. D. McLachlan, personal communication). The axis of this symmetry is along the centre of the four-stranded supercoil and approximately through the centre of the β sheet. Variation in the character of the protein along the direction of this axis reflects a segregation of function. Nearest to the centre of the disk is the flexible loop, awaiting interaction with RNA. Next comes the α-helical core and β sheet providing rigidity within the subunit while allowing flexibility across the interface. Last, at the distal end of the subunit is the hydrophobic girdle extending around the entire ring.

The discovery of the flexible loop in our 5 Å map[5] gave insight into the means whereby TMV protein can interact with, in effect, a random RNA sequence in the complete virus, and yet also recognise the special nucleation sequence of TMV RNA[7]. The binding site must combine the specificity required for the initial recognition of the special sequence with the ability to accommodate subsequently any sequence of RNA. Stereospecificity will be provided by the rigid parts of the protein in the vicinity of the RNA binding site: the proximal ends of the RR and LR helices and the hairpin bend, joining the proximal ends of the two slewed helices, which forms a rigid wall (with a concentration of invariant residues) facing into the cavity of the disk. Preliminary evidence suggests that nucleotides bind below the RR helix[28].

The mobility of the flexible loop in the disk is reminiscent of that found in the activation domain in trypsinogen[30]. Segments from four separate elements of this chain are flexible in the zymogen but become tightly interdigitated in the enzyme, after cleavage of the activation peptide and associated conformational changes, forming a rigid, correctly defined specificity pocket for binding the substrate. Creation of the binding site in TMV does not involve protein cleavage, as observed in trypsin, but it may be noted that when such cleavage does occur in the flexible region of disks of TMV it is essentially nonproductive, leading irreversibly to the stacked disk aggregate[31] of TMV protein which is not capable of initiating the assembly process.

The strong tendency of TMV protein to aggregate[1] can now be understood in terms of its surface properties. The hydrophobic nature of both the centre of the supercoiled bundle formed by the four α helices and the aromatic cluster in the centre of the subunit on the opposite side of the β sheet are not unusual, but the hydrophobic patches on the molecular surface would only be expected in a component of a larger assembly, not in a monomeric protein. These patches occur only within the lateral interface, which is essentially the same in the disk and the virus. This interface comprises hydrophobic and salt interactions which would both be favoured by higher temperatures, and indeed, TMV is a classical example of an entropically driven system[32]. The general character of the alternating hydrophobic and salt-bridge patches within this interface is conserved in all strains of the virus which have been sequenced, although the boundaries of the patches differ slightly between strains. A fuller discussion of this will be presented elsewhere.

The salt-bridge systems within the lateral interface remain on the transition from disk to virus, but the most extensive system, that between the layers of the disk, is completely disrupted by the transition, which changes the relative positions of subunits in the two layers both axially and azimuthally[5]. The interaction between subunits in successive turns of the virus places the first turn of the RR helix, which is the lowest part of the subunit in the A ring of the disk (Fig. 4), in the groove between the distal ends of the LS and RS helices.

The implications for virus assembly of this rearrangement were discussed in our previous paper[5]. The prediction that RNA interacts with TMV protein from the centre of the disk has been verified with the discovery of a hairpin loop structure[8] for the nucleation sequence within the RNA[7] and the observation that both tails of the RNA emerge from the same end of the growing rodlet[33,34]. Insertion of this loop into the central hole of the disk[6], and incorporation of RNA between the two layers, resolve the paradox posed by this two-layered structure. There is now no need to postulate separate mechanisms for the initiation and elongation stages of virus assembly. A travelling loop within the RNA which starts at the nucleation sequence for the initiation process and moves towards the 5′-end during elongation, uses the same forces of interaction for both processes. An understanding of this interaction in atomic detail must await the correlation of our model for the protein with the high-resolution fibre-diffraction pattern of the virus. This study is now in progress (in collaboration with Dr G. J. Stubbs).

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Tomato bushy stunt virus at 2.9 Å resolution

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The polypeptide chain of a TBSV subunit folds into two domains, connected by a hinge, and a flexibly-linked N-terminal arm. Sixty of the 180 N-terminal arms interdigitate in groups of three, in an unexpected mode of protein association. The remaining 120 arms are not uniquely positioned with respect to the rest of the subunit. RNA is also not uniquely fixed to sites on the major domains.

SMALL, regular viruses self-assemble in solution[1,2]—implying that the protein subunits can associate correctly without further direction and that they can package nucleic acid. In most spherical viruses, correct assembly requires that a single type of subunit be capable of several, somewhat different bonding relations ('quasi-equivalence'). The structure of tomato bushy stunt virus (TBSV), as it appears at 5.5 Å resolution[3], shows that its subunit (MW ~ 40,000) achieves such flexibility by having two rigidly-folded domains with an interconnecting hinge. Two states of the hinge, differing by about 20°, are present in the $T=3$ (180-subunit) icosahedral structure. A large number of inter-subunit contacts are conserved in this way, maintaining specificity.

The low-resolution view of TBSV left a number of questions unanswered. How is the hinge flexibility controlled and a particle of correct size generated? With what precision are inter-subunit contacts conserved? How is RNA packed? To explore these questions, we have extended the resolution of the TBSV structure determination to 2.9 Å. We present here a first description of the results, which show several remarkable features. Near the N terminus of the polypeptide chain, 60 of the 180 chains are ordered, interdigitating in groups of three, whereas the remaining 120 extend in a less precise way towards the particle centre. Moreover, RNA does not seem to bind to the part of the subunit rigidly fixed in the surface lattice, and the structure suggests that the N-terminal arm, flexibly-linked to the rest of the subunit, has an RNA-binding function.

Structure determination

TBSV crystallises in the cubic space group I23, $a=383.2$ Å (refs 3–5). Diffraction to 2.85 Å was measured by oscillation photography[6], with a $\frac{1}{2}°$ range for each picture and with a total oscillation range of 25° about [110] required for a full data set. By translating a crystal between exposures, we could generally obtain two good photographs, and we therefore needed about 25 crystals for complete data. The films were scanned using a modified version of SCAN 12[7] to control an Optronics Photoscan interfaced to a PDP 11/20. As about 75% of the reflections were partially recorded, and as the crystal lifetime in any one location did not permit summing intensities of complementary spots on adjacent films in a series[6], a strategy for correcting to fully-recorded equivalents was adopted: details of the method will be published elsewhere (F.K.W. and C.E.S., in preparation). Corrected intensities were included in the final data set from only those reflections on any film that were at least half-recorded: by taking photographs with approximately abutting oscillation ranges, essentially all reflections were measured on one film or the other. The accuracy of measurements corrected in this way is comparable to the accuracy of fully-recorded reflections of the same observed strength.

Three data sets were collected: native and two derivatives (UO_2^{2+}, Hg^{2+}). The complete native data set contained 198,000 reflections: some statistics are given in Table 1*a*. Results of the heavy-atom refinement are shown in Table 1*b*. Phase determination by non-crystallographic symmetry averaging[8] proceeded in a manner similar to the phase refinement at 5.5 Å (ref. 3). A full account of the calculations, carried out at the CECAM Workshop on Virus Crystallography, will appear elsewhere (G.B., in preparation). A brief summary is included in Table 1*b*. The Fourier map used for averaging[8] was computed on a 0.6 Å grid from 182,000 terms between 16 and 2.9 Å; it had 37×10^6 points in an I222 asymmetric unit. The map computed after four cycles of phase refinement was used for the computation of

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Table 1 Details of the structure determination of TBSV

a. Data collection:

Form	Reflections measured	R
	6 Å → 2.89 Å	
Native	182,000 (plus 17,000 to 6 Å from earlier work)	0.13
UO_2^{2+}	164,000	0.14
Hg^{2+}	130,000	0.15

$$R = \frac{\sum_h \sum_{j=1}^{N_h} |I_{hj} - \bar{I}_h|}{\sum_h N_j \bar{I}_h},$$ where the reflection, h, has been measured N_h times in data collection, and the average and differences are taken after appropriate scaling, and

$$\bar{I}_h = \sum_{j=1}^{N_h} W_{hj} I_{hj} \Big/ \sum_{j=1}^{N_h} W_{hj}$$ where $W_{hj} = 1/\sigma^2(I_{hj})$. The mean value of N_h for the native data set was 3

b. Phase determination:

Heavy-atom parameters (re-refined with phases after one cycle of noncrystallographic-symmetry phase refinement, using selected well-measured reflections; see ref. 3).

UO_2^{2+}						Hg^{2+}					
Site	F	B	x	y	z	Site	F	B	x	y	z
U A1	83	22	−0.0446	0.0708	0.3363	H A1	78	20	−0.0377	0.0896	0.3336
U B1	74	21	0.0225	0.1082	0.3383	H B1	91	16	0.0357	0.0931	0.3340
U C1	81	21	0.0225	0.0304	0.3364	H C1	74	21	0.0019	0.0271	0.3350
U A2	91	19	−0.0147	0.0979	0.3477	H A2	37	26	−0.0677	0.1208	0.4233
U B2	84	18	0.0316	0.0676	0.3471	H B2	39	27	0.0815	0.0977	0.4226
U C2	43	27	−0.0171	0.0437	0.3458	H C2	37	26	−0.0150	0.0151	0.4307
U C3	44	15	0.0172	0.0261	0.3500						
			$\langle F_{nat}\rangle = 84.3$						$\langle F_{nat}\rangle = 84.3$		
			$\langle \Delta F\rangle = 16.3$						$\langle \Delta F\rangle = 13.9$		
			$R_c = 0.655$						$R_c = 0.662$		
			$\langle F_c\rangle/E_{r.m.s} = 1.71$						$\langle F_c\rangle/E_{r.m.s} = 1.38$		

R_c = Cullis R factor for centric reflections

$\langle F_c\rangle/E_{r.m.s}$ = 'phasing power' (mean calculated F divided by r.m.s. lack of closure)

Noncrystallographic-symmetry phase refinement[8]

Cycle	R	$\langle\lvert\Delta\phi\rvert\rangle$	m_{MIR}	m_{Sim}	m_{comb}
1	0.41	53°, 33°*	0.46	0.56	0.70
2	0.30	25°			
3	0.23	8.5°			
4	0.20	5.1°			

* Cycle 1 was performed with phase combination; the mean $\Delta\phi$ between MIR and calculated phases was 53°, the mean $\Delta\phi$ between MIR and combined phases was 33° Subsequent cycles were carried out with direct phase transfer.

R: $\frac{\sum \lvert\, |F_{calc}| - |F_{obs}| \,\rvert}{\sum |F_{obs}|}$, where F_{calc} is the amplitude calculated from a symmetrised map at each stage, and F_{obs} is the measured amplitude

$\langle\lvert\Delta\phi\rvert\rangle$: mean phase change

m_{MIR}, m_{Sim}, m_{comb}: mean figures-of-merit for cycle 1

icosahedrally averaged maps of individual subunits, using a generalised skew planes procedure[8].

In considering the effects of icosahedral averaging, both in phase refinement and in examining the final maps, it is important to recall that the TBSV particles lie at positions of tetrahedral symmetry in the crystals. There is no chemical evidence for 'twelveness' in the particle—that is, no evidence for any special role for the tetrahedral subgroup of symmetry operations[9]. Therefore, the fivefold averaging imposed by the calculation is not 'smearing out' any structures (such as RNA or the possible unique subunit) that have not already been averaged out by the space group itself. Tetrahedral distortions of the particle, imposed by the lattice contacts, are of course always possible. But a difference map using $(|F_{obs}| - |F_{calc}|) \times \exp(i\alpha_{calc})$ coefficients, 'calc' referring to the last cycle of icosahedral averaging, showed no significant features; even near interparticle contacts. Such distortions must therefore be extremely small.

Maps of subunits in quasi-equivalent locations A, B and C (Fig. 1) were prepared, sectioned such that all three domains appear in the same orientation, using superposition parameters determined from the 5.5 Å map[3]. This presentation facilitates visual comparisons. Features in these averaged maps are extremely clean and well defined (Fig. 2): the polypeptide chain can be traced unambiguously, side chains are well-outlined, and main-chain carbonyls are visible for most residues. The structural results to be presented below are all derived from inspection and measurement of mini-maps (3.2 mm per Å) contoured from these averaged subunit densities. Model-building with an optical comparator and Kendrew parts is in progress.

Organisation of protein shell

The packing of protein subunits in TBSV is schematically illustrated in Fig. 1. The icosahedrally-ordered parts of the virus particle can be described as a compact polyhedral shell, about 30 Å thick, with 90 projections at strict and local twofold positions[3]. This structure is achieved by regular interactions of subunits that fold into two distinct globular domains, connected

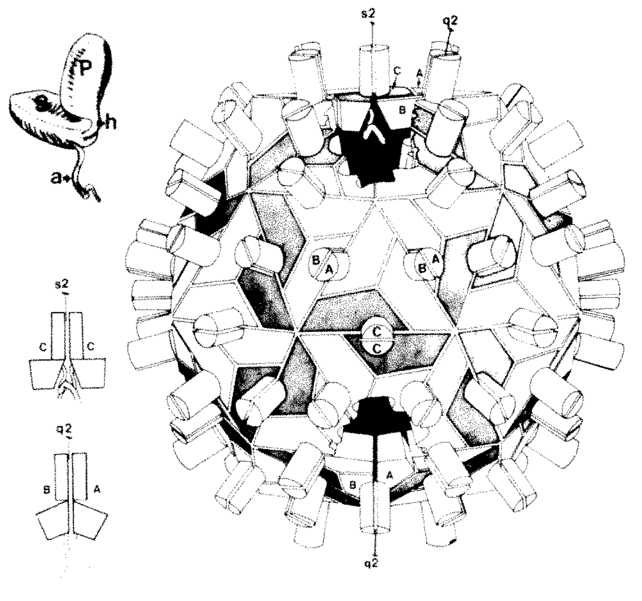


Fig. 1 Packing of protein subunits in TBSV. *a*, Schematic subunit, showing P and S domains, the inter-domain hinge (h) and the N-terminal arm (a). *b*, Arrangement of subunits in the virus particle. A, B and C denote the three packing environments of the subunit; outer surfaces of C subunit S domains are shaded. S domains of A subunits pack around fivefold axes; S domains of B and C alternate around threefolds. The quasi-threefold axis relating S domains of an ABC trimer is nearly parallel to the adjacent strict twofold (s2). Trimers therefore present a rather flat surface across the strict dyad and a distinctly sharper dihedral angle (about 40°) across the quasi dyad (q2). This difference in local curvature can be seen at the two places where the shell has been cut away to reveal S domain packing near strict (top) and quasi (bottom) dyads. *c*, The two states of the TBSV subunit found in this structure, viewed as dimers about strict (s2) and local (q2) twofold axes. Subunits in C positions have the inter-domain hinge 'up' and a cleft between twofold-related S domains into which fold parts of the N-terminal arms. Subunits in the quasi-twofold-related A and B positions have hinge 'down', S domains abutting, and a disordered arm.

by a hinge. We indicate these domains by the letters P (projection) and S (shell)—corresponding respectively to domains 1 and 2 in our previous paper[3]. The 2.9 Å electron density map shows that these domains include only about three-fourths of the sequence, and that the remainder of the chain forms an inward-projecting N-terminal arm. If we denote the three subunit packing modes by A, B and C (compare Fig. 1), then the overall organisation of the virus particle can be summarised as follows. The globular P and S domains fold in an essentially invariant manner. There are two positions of the inter-domain hinge—the A/B configuration (subunits related by a local twofold) and the C configuration (subunits related by a strict twofold), with a relative reorientation of about 20° in passing from one to the other. The A and B backbone conformations and hinge positions are indistinguishable at present, but some differences in side-chain conformations are evident. The conformation of the arm differs radically in position C from its conformation in A or B. Indeed, the map shows no density for the arm on subunits A and B, implying that it is not positionally fixed to the main body of the subunit. On C, however, the arm folds back along the edge of the S domain and interdigitates with two other such arms around the particle threefold axis, forming a remarkable structure described in more detail below. The folding of this part of the subunit depends on its contacts with several other subunits. These interactions seem to lace the particle together in a manner quite different from the cohesion provided by contacts between the independently folded globular domains.

Chain trace

The entire ordered part of the polypeptide chain has been traced without ambiguity (Fig. 3). At a few points, especially near heavy-atom sites, it was helpful to compare subunits in locations A, B and C (because we expect noise and artefact to be different in each), but in general each chain could be followed independently. Polarity was established initially by identification of carbonyls in the one α-helix, and confirmed by the orientation of carbonyls and C_β in β-sheets, by the handedness of β-sheet crossovers and so on.

The N terminus is in the particle interior; the C terminus, on the outside. The sequence of domains along the chain (N-to-C) is therefore: arm, S domain, P domain. The P domain comprises about 110 residues; the S domain, 168. The portion of the N-terminal arm on subunit C that is visible in the map contains

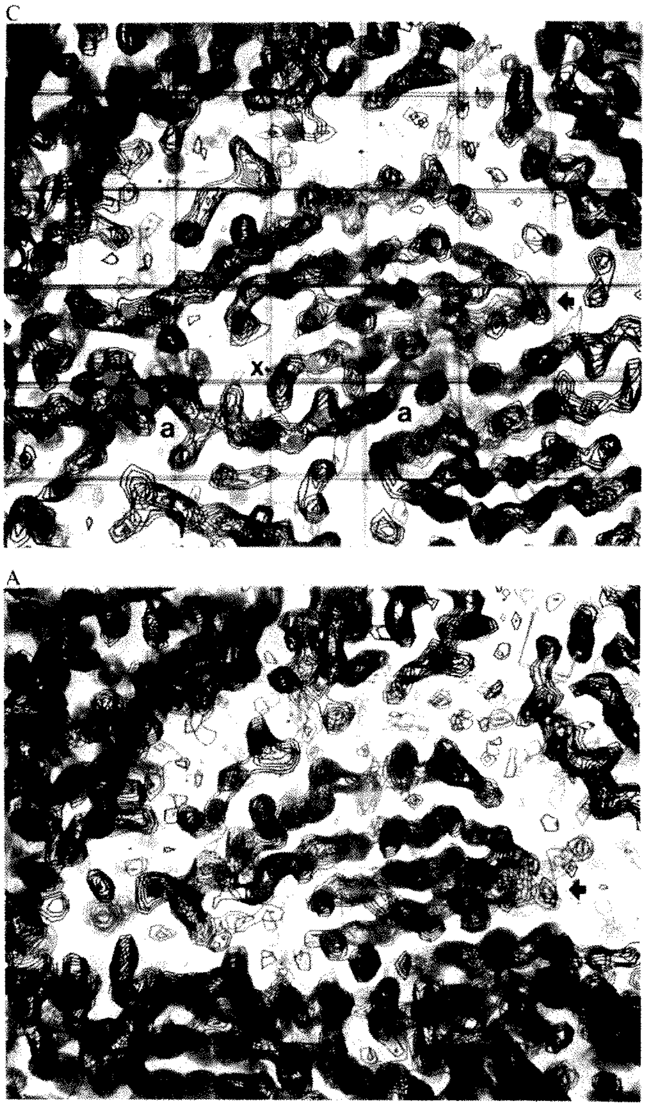


Fig. 2 Portions of the icosahedrally averaged final 2.9 Å electron density map of TBSV. The views are of the inward-facing surface of S domains of subunits A and C, as seen looking down a twofold axis (subunit C, oriented as shaded unit in Fig. 6), or quasi-twofold axis (subunit A). The sectioning of subunit A has been chosen to correspond as closely as possible to the sectioning of subunit C: the invariant fold of the S domain is evident from the comparison. Grid squares correspond to 10 Å. This part of the S domain is a four-stranded β sheet, with the arm (a) folded back alongside it in the case of C. The periphery of each photograph includes features from adjacent subunits, so that this part of the density appears different in each view. Note the large side chain (x), hydrogen-bonded to a backbone carbonyl of the arm of C and disordered on A. Bold arrows show the point where chain folds back into arm on C and extends more irregularly into the centre on A.

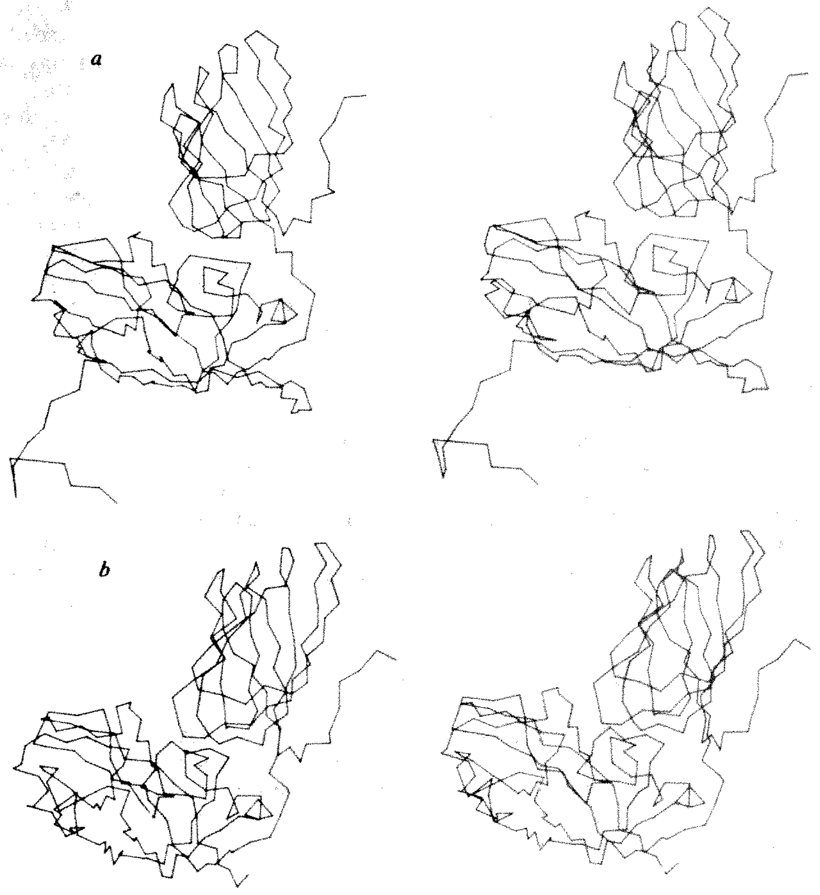


Fig. 3 Stereo views of the two states of the TBSV subunit: (*a*) C, with ordered arm and hinge 'up'; (*b*) A/B, with arm not fixed (and hence not shown) and hinge 'down'. The view of C is normal to the twofold axis, in the direction of the arrow in Fig. 6; the view of A is chosen such that S domains are as closely superposed as possible. The line segments making up these drawings join α-carbon positions, indicated by their intersection. The α-carbon coordinates were measured from mini-maps as described in the text.

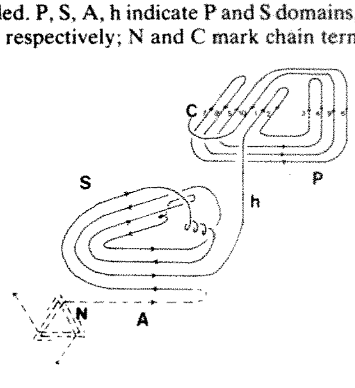
33 residues. The total of 311 identifiable residues corresponds to a molecular weight of ~34,000 (taking 110 as a mean residue weight). As the polypeptide chain molecular weight determined by gel electrophoresis and by gel filtration in guanidine hydrochloride[9,10] is 40,000 ± 2,000, there may be as many as 50 or 60 additional residues, not visible in the map even in subunit C. The connectivity of P and S domains and of the arm is clear. The C terminus also appears to be very clear: moreover, the position of specific sulphydryl cleavages as well as the remarkable protease resistance of the intact particle (A. Andreadis and S.C.H., unpublished) argues strongly against large 'dangling' loops at the outside. The most likely explanation for any difference between the number of visible residues and the expected total is therefore a further segment at the very N terminus. The complete N-terminal arm could then contain as many as 80–90 residues, all of them disordered in positions A and B, and the 33 closest to the S domain ordered in position C. Since the amino acid sequence is unknown, a more exact estimate cannot yet be made. We emphasise that the absence of density corresponding to N-terminal segments does not imply the absence of a regular fold for this part of the polypeptide chain; it requires merely that the terminus not be fixed spatially in the same way on all symmetrically related subunits.

The P domain is composed of two entirely antiparallel β sheets, one of six strands and the other of four (Fig. 4). The six-stranded sheet lies adjacent to a twofold axis and therefore packs against a similar sheet in the related subunit. The curvature of the four-stranded sheet is such that towards the particle centre (bottom of the domain in Fig. 3) it lies at some distance from the six-stranded sheet, with the strands curving gently around from one sheet into the other while preserving a β hydrogen-bond relationship. The two sheets are thus partly continuous along the chain direction (strands 4 to 5, 6 to 7, 8 to 9), although there appears to be no main-chain hydrogen bonding across the ends of the 'sandwich' (that is, between strands 2 and 3 or 6 and 7). About 20 residues at the C terminus form a strand that curls under the domain and up to the terminal residue, which lies at the subunit interface.

The S domain (Fig. 4) is also largely β sheet. It is shaped roughly like a triangular prism; a bent, four-stranded sheet forms the lower surface and one side of the most acute angle of the prism; a second four-stranded sheet forms the other side of the angle. The opposite face, and the upper surface, are formed by loops and by two short helical segments. The longer of these helices (about $2\frac{1}{2}$ turns) is tangentially directed and lies adjacent to a dyad; the other is a quite irregular helix of less than two full turns, radially directed, and packed against a sheet. The loops and helices appear to contain those residues most critically involved in variable inter-subunit or inter-domain contacts (compare 'contacts', below). In subunit C, the N-terminal arm folds back along an inner edge of the prism-shaped domain, forming a fifth element of one of the β sheets.

The inter-domain hinge consists of just two or three residues, with the backbone well ordered in each of the two observed configurations. There are probably small changes in several backbone torsion angles generating the hinge displacement.

Fig. 4 Schematic representation of the TBSV polypeptide chain, showing connectivity of domains and secondary structure within them. Lines with arrows indicate strands of β-pleated sheet (all antiparallel in this case). The N-terminal arm and β-annulus are shown by dashed lines, with segments of the two other chains in the annulus included. P, S, A, h indicate P and S domains, the arm and hinge respectively; N and C mark chain termini.

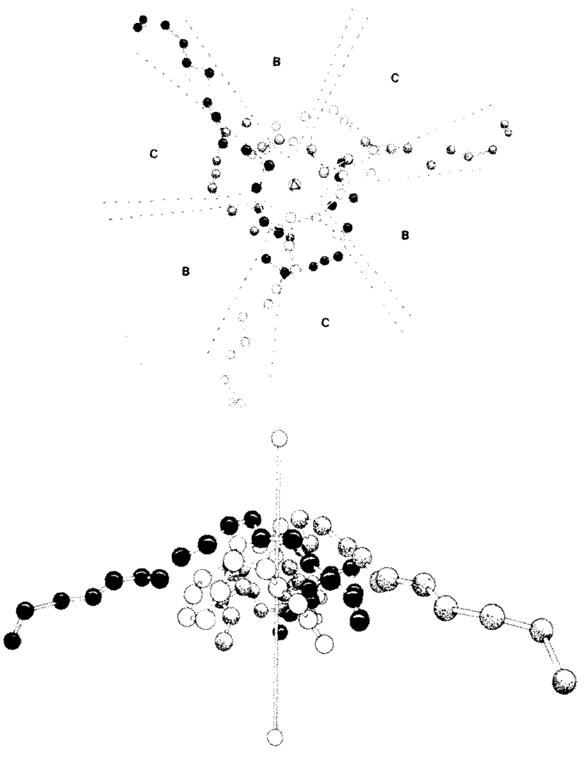


The chain trace described above exhausts all strong features of the map. Additional parts of the polypeptide chains, and all the nucleic acid, must be averaged over a number of orientations with respect to the principal domains. Density ascribed to RNA in interpreting the 5.5 Å map of TBSV ('*e*' in Fig. 2*b* of ref. 3) is now clearly seen to be the arm of subunit C.

N-terminal arm

The most remarkable aspect of the TBSV subunit is the configuration of its N-terminal portion. Following the chain in the C-to-N direction through the S domain in subunits A and B, we observe that the strong, distinct features in the map 'fray-out' and weaken at residue '35', and that any clear chain trace is impossible after about residue '33'. (The numbering scheme adopted here begins at the first visible residue on subunit C. Residue '1' may be up to 50 residues in from the actual N terminus: see preceding section.) By contrast, in subunit C, the chain executes a reverse turn and folds along the inner edge of the S domain next to the twofold axis. Its subsequent course is shown in Fig. 5. Extending towards the particle threefold axis, in the cleft between adjacent S domains, the chain makes a number of contacts with the neighbouring (B-type) subunit. On reaching the region of the threefold axis it winds anticlockwise around the axis (viewed from outside the particle), and inwards, forming a roughly 240° circuit from residues '18' to '1'. As there are, of course, two other symmetry-related chains, the 54 residues form an annular structure around the threefold axis. Their configuration is rather like the overlapping flaps of a cardboard carton: chain 1, over chain 2, over chain 3, over chain 1. This threefold ring seems to be stabilised by several sorts of interactions. Residues 3–5 of one chain, 11–13 of a second and 16–18 of a third form a little three-residue, three-strand β sheet: each chain enters such a sheet three times, once at each level, and the entire structure might well be called a 'β-annulus'. In addition to these main-chain interactions, there are important side-chain contacts. Residues 4 and 15, for example, have side chains that make contact around the threefold, partly defining the 'inside' of this small, globular cluster. Moreover, the entire cluster is suspended inward from the apices of the six neighbouring S domains (three C types, corresponding to the subunits to which the three participating arms 'belong', and three alternately intervening B types).

Fig. 5 Configuration of the β annulus, composed of three interdigitating arms. Balls show α-carbons; sticks are schematic indications of the peptide-chain connection. (*a*), View down threefold axis; (*b*), view normal to threefold axis.



It should be observed that, although the interdigitation of arms in the β annulus is at first sight complex, it presents no problem in principle for folding. No knots are implied, for example, and the structure can form by the interweaving of loops in a manner consistent with the presence of further chain distal to it. Nonetheless, this folding must occur during assembly, since the chain depends for its configuration on interaction with a number of neighbouring subunits. Indeed, the precise final organisation of the arm is clearly possible only when B-type subunits are present as well. In this respect, the arm of the TBSV subunit is similar to the N-terminal arm of LDH[11] and to crystalline glucagon[12], where acquisition of the observed conformation seems to depend on formation of the final assembly.

This map does not show the conformation of the arm of subunits A and B, nor that of any additional subunit C residues beyond the annular structure just described. The absence of strong density corresponding to these portions can have several possible interpretations. The segments in question could adopt more than one orientation from subunit to subunit. The map would then have a 'smeared-out' superposition of these alternative structures. The disorder could also be dynamic with all or part of these segments undergoing rather large-scale diffusional motion. Preliminary NMR measurements (C. Dobson and S.C.H., unpublished) lead us to prefer the former picture.

Subunit contacts

Individual domains are identically folded in all three quasi-equivalent subunit locations. We have not yet analysed intra-domain flexibility in detail, but its magnitude is relatively small (C_α superpositions of individual domains give r.m.s. differences of 1 Å or less, the expected measurement error). Variation is concentrated at the domain interfaces, both within and between subunits, and restricted to alternative positions of side chains rather than of the backbone. We can obtain a first description of the capacity of a TBSV subunit for variable packing by examining the degree of conservation or nonconservation of side chain conformation and interaction at the domain interfaces.

Each local symmetry element gives a class of subunit contact (Fig. 6). These contacts may be further subdivided according to the domains involved. The analysis is summarised in Fig. 6. In general, it seems that contacts are either fully conserved (identical side chain packing at quasi-equivalent positions) or distinctly altered. The fivefold/sixfold contacts are a good illustration. These contacts are 'quasi-equivalent' in the sense that they involve the same parts of the subunit. 'Sixfold' is something of a misnomer, however, except in the sense of coordination. As shown already at 5.5 Å, pairwise contacts of the six S domains packed about a strict threefold axis alternate in character[3]. Proceeding anticlockwise (viewing from outside the particle), C → B contacts are essentially identical to contacts about a fivefold axis, while B → C contacts involve quite different interactions, due to intervention of the C subunit arm. At these, as at other non-conserved interfaces (S domain twofold contacts; P domain/S domain contacts), side chains that make important interactions in one state often appear rather disordered in the other.

The conserved local-threefold interfaces have one particularly interesting feature—an ion, perhaps calcium, coordinated by side chains from both subunits. This site is probably linked to an EDTA-induced swelling observed at *p*H 7.5 (S.C.H. and A.J.O., unpublished).

RNA

There is no strong density in this map that can be ascribed to RNA; the entire inner cavity of the virus particle ($r < 112$ Å) is devoid of dense or interpretable detail. This cavity must, however, contain both the nucleic acid molecule and the protein

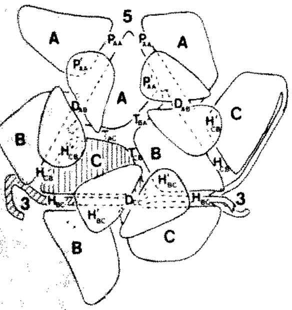


Fig. 6 Schematic diagram showing subunit packing and contacts. The diagram is effectively a more detailed view of the lettered subunits seen nearly face-on in Fig. 1. The domains are indicated in rough outline: barrel-shaped P domains protrude outwards, making only twofold contacts, while the more extensive S domains make contacts across all adjacent symmetry axes. The arms of subunits C fold back as indicated; the arms of A and B are not fixed. The shading of one subunit C indicates which P domain (stippled) is associated with the labelled S domain (hatched). Contacts between subunits are lettered to provide a key for the Table. The cation sites (see text) are near the letters 'T'.

Conservation and shift of inter-subunit contacts

Contact	Code in Figure	Packing
P domain/P domain		
Twofold	D_{CC}	Conserved (apposed β-sheets)
Quasi-twofold	D_{AB}	
S domain/S domain		
Twofold	D_{CC}	Non-conserved (Helices roll over each other; sheet edge contacts displaced by arms)
Quasi-twofold	D_{AB}	
Quasi-threefold	T_{AC}, T_{CB}, T_{BA}	Conserved (Ca^{2+} site)
Fivefold	P_{AA}	Conserved
Quasi-sixfold (fivefold-like)	H_{CB}	
Quasi-sixfold (across cleft)	H_{BC}	Non-conserved (Displaced by arm)
P domain/S domain		
Fivefold	P'_{AA}	Conserved
Quasi-sixfold (fivefold-like)	H'_{CB}	
Quasi-sixfold (across cleft)	H'_{BC}	Non-conserved (Different residue of P domain contacts top of short radial helix)

arms. The volume available to RNA, allowing for the arms, is about 5×10^6 Å^3. This corresponds to about 1.5 g H_2O per g RNA—equivalent to the hydration of DNA in phage heads[13] or to the solvent content of orthorhombic yeast-phenylalanine-tRNA crystals (about 1.3 g per g, ref. 14). TBSV RNA is therefore very tightly packed.

Small angle X ray[15] and neutron scattering from TBSV in solution (B. Jacrot, personal communication) indicate that RNA is present at radii immediately internal to the S domains seen in the present map. We have considered whether dissociation of RNA from fixed binding sites on this domain might have been produced by the relatively high ionic strength (~1.2 M) required for crystallisation. We have succeeded in obtaining high-resolution X-ray photographs from TBSV crystals in 0.1 M $(NH_4)_2SO_4$, 20% polyethylene glycol (ionic strength ~0.3 M): no significant intensity changes are observed at any resolution. Moreover, we note that the inward-facing part of an S domain presents a relatively smooth surface to the interior, unlike the clefts between distinct lobes that characterise the active sites of catenases such as lysozyme and ribonuclease[16]. We conclude that equivalent subunits do not necessarily 'see' RNA in the same position or orientation, thus accounting for the absence of strong high-resolution features.

How, then, does RNA interact with the TBSV subunit? The arguments just outlined imply that the arms of subunits A and B, and the presumed N-terminal part of the arm of C, are interspersed in some way with RNA. Detailed interpretation of neutron scattering suggests considerable penetration of protein towards the particle interior (B. Jacrot, personal communication). We have at present no direct evidence for specific interaction, but the arms are obvious candidates for protein-RNA binding. An RNA-binding region adopting various positions with respect to the body of the subunit might find good contacts, whatever the detailed three-dimensional arrangement of RNA; it would thereby gain the advantage of relative insensitivity to nucleotide sequence.

Implications for assembly

The 2.9 Å map has revealed two quite unexpected aspects of the TBSV structure. A portion of the polypeptide chain near the N terminus interdigitates with others in an unusual fashion in one state of the subunit (C) and appears disordered in the other, quasi-equivalent positions (A/B). Moreover, RNA appears not to bind to the rigidly-anchored S domain; it may instead interact with the flexibly-linked arms. Some interaction of RNA and protein is required for self-assembly.

We have as yet no information about the pathway of TBSV assembly, but we would like to draw attention to one striking feature of the particle design. The threefold-linked arms, together with the D_{cc} contacts, interlace subunits in such a way that all sixty C type subunits are interconnected, without mediation of A or B (see Figs 1, 6). That is, removal of all A and B subunits would leave a still intact, though fenestrated and cage-like, shell. Moreover, all subunits in this shell would be exactly equivalent in conformation and bonding. Twofold (P domain) and threefold (β annulus) contacts in such a structure would determine completely a 60-subunit framework, though perhaps a rather floppy one, connected like the edges of a pentagonal dodecahedron. This hypothetical structure illustrates that no 'switch' is needed to control hinge configuration or size determination. With C subunit arms linked to form a β annulus, the intervening gaps can be occupied only by subunits that adopt an A/B conformation, having hinges 'down' and unlinked N-terminal arms. That is, if linkage of the arms is important in building up a particle, non-equivalent aspects of subunit packing are automatically built into the bonding scheme. Moreover, as A/B subunits are introduced into a network of C's, the structure acquires the rigidity and curvature of a complete T = 3 virus shell.

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letters

Incidence of ionised gas in E/SO radio galaxies

SEVERAL nearby E and SO galaxies are known to contain weak ($L_{\rm rad} \sim 10^{39}$ erg s^{-1}) radio sources[1-3]. Heeschen[4] found that these sources fall into two distinct classes: 'extended' sources with steep radio spectra and sizes comparable to their parent galaxies, and 'compact' sources with flat spectra, which are centred on the optical nucleus and have sizes smaller than a few arc s. Although ionised gas is generally inconspicuous in E/SO galaxies, spectroscopy of ~24 E/SO radio sources[3,5,6] suggests a high incidence of optical emission lines in galaxies with compact radio structure. The two best-known examples, NGC1052 and NGC4278, have, in addition to unusually strong emission lines, abnormal amounts of neutral hydrogen[7-9], and this has led to the speculation[10] that radio outbursts in early type galaxies are fuelled by accretion of neutral gas, possibly from intergalactic clouds. As part of an optical study of E/SO sources with flux densities higher than 20 mJy from the deep Arecibo radio survey of Dressel and Condon[11], we have recently obtained spectrophotometry of 57 objects which makes the radio structure–optical line emission correlation more definitive. The 2,380 MHz Arecibo survey of bright Uppsala General Catalogue galaxies included 2,095 objects with $0° \leq \delta \leq +37°$. 24% of the E, and 12% of the SO galaxies were detected at the 5σ level ($S_{2380} > 15$ mJy). Interferometer observations[12] of the brighter sources allowed separation into the compact (CRS) or extended (ERS) categories; continuing work with higher resolution indicates that several ERS objects have a well-defined double structure (J. J. Condon, personal communication). The optical observations were made with the image dissector scanner on the Kitt Peak National Observatory 2.1 m telescope in December 1977. The scanner was operated with an 8.4-arc s diameter entrance aperture, which yielded an effective resolution of ~11Å. Scans covered $\lambda\lambda$ 3400–5200. We confine our attention here to [O II] λ 3727, which is expected to be ~10 times stronger against the galaxy background in objects with spectra like NGC1052 (ref. 8) than is Hβ.

The limiting detectibility for our discussion is $W(3727) \sim 0.6$ Å equivalent width. A more refined analysis which includes comparison to line-free absolute energy distributions should yield a limit of ~0.2 Å. For comparison, the limit for [O II] observations by Humason, Mayall and Sandage[13] is ~10 Å. Our observations included all Arecibo E/SO detections with $0\,{\rm h} < \alpha < 11.7\,{\rm h}$ or $20\,{\rm h} < \alpha < 24\,{\rm h}$ and with $S_{2380} > 30$ mJy, as well as five fainter detections. Additional CRS objects were selected from lists of sources outside the Arecibo declination limits. Finally, a selection of non-radio source (NRS) E/SO galaxies, with $S_{2380} \leq 3$ mJy was included from the Arecibo survey as a control group. Objects with morphological classifications later than SO in the reference catalogue[14] are excluded.

Results are shown in Fig. 1, which plots $W(3727)$ against a radio-optical flux ratio defined as $R = 10^{-5}\, S_{2380}/10^{-0.4m}$, where m is a corrected apparent magnitude described in ref. 12. Apart from the three Seyfert galaxies, two of which (UGC3374 and 5025) are newly identified Seyfert 1 objects, rich emission line spectra were not encountered. (The Seyferts are probably spirals, not E/SO galaxies[15].) Figure 1 also shows a histogram of line strengths in NRS objects. Only 4, or 20%, have detectable λ 3727. For the ERS objects, only 4 out of 16 were detected, and one of these has a compact core component contributing 20% of the total flux. The two strongest ERS [O II] detections (and two of the four NRS cases) exhibit evidence of young stellar populations, and their line emission may be unrelated to their radio properties. In contrast, all 12 Arecibo CRS objects and four out of six non-Arecibo CRS objects were detected. Most of these have $W(3727) > 10$ Å, whereas only one ERS/NRS detection is this strong.

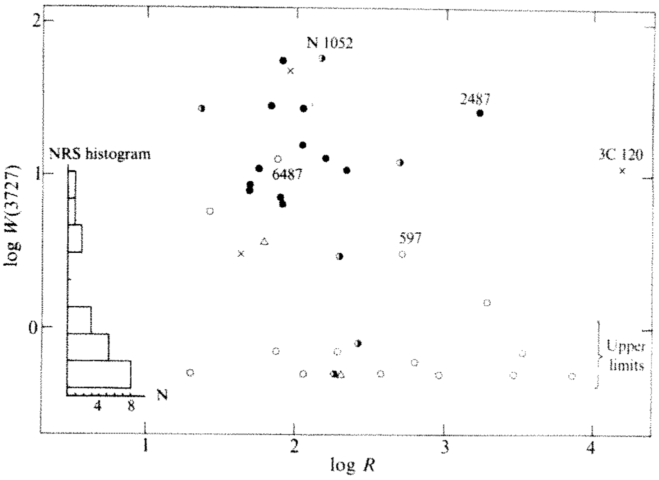

Fig. 1 A plot of the [O II] equivalent width against a radio–optical flux ratio described in the text. Strengths < 1 Å are upper limits. At the left is a histogram of [O II] strengths in non-radio source objects, for all of which $R < 6$. CRS objects have > 50% of their radio flux originating in a region < 6 arc s in diameter. The notation ERS + CRS indicates objects with distinct core components which contribute < 50% of the flux. Labels without prefixes are UGC numbers. ○, ERS; ●, CRS; ◑, CRS, non-Arecibo; △, ERS + CRS; ×, Seyfert.

We thus conclude that among E/SO galaxies, CRS objects have a high probability of strong [O II] line emission whereas ERS objects, despite R values which are typically an order of magnitude higher, are no more likely to exhibit [O II] than galaxies without detectable radio emission. Because most CRS objects are SO galaxies while most ERS objects are E galaxies[12], it is important that this relationship holds for E or SO systems considered separately, although the samples in each category are small. On the basis of these results, one expects that a large fraction of E/SO galaxies with [O II] detected by Humason, Mayall and Sandage[13] will turn out to have compact radio cores. There is no obvious correlation in Fig. 1 between [O II] strengths and the luminosity of the compact source, suggesting that photoionisation by non-thermal radiation may not be responsible for heating the gas.

Because compact radio sources require replenishment of relativistic electrons on a much shorter time scale than extended sources, it is reasonable to expect a correlation like that observed here if infall of thermal gas on to a condensed nuclear object is responsible for the production of relativistic plasma. It now seems that such gas in most cases must originate inside the

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parent galaxy, as the lack of intergalactic H I clouds[16,17] together with the fact[12] that CRS objects are not preferentially found in galaxy groups or clusters argues that accretion of external gas is rare and probably unnecessary for CRS production. Rather special conditions are required for accretion of hot intracluster gas[18]. Gas lost during normal stellar evolution can readily meet the power requirements of these sources if it is acquired by an accretion disk surrounding a massive black hole.

The high incidence of line emission in CRS objects does not, however, prove that gas infall fuels radio outbursts. The compact source may merely provide a heating mechanism for small amounts of neutral gas (say $\sim 10^6 M_\odot$, which is impossible to detect with current 21-cm sensitivities[7]) which are incidentally present in E/SO galaxies. In ERS objects, emission might be suppressed through stripping of the thermal gas by an external medium (as most ERS sources apparently reside in clusters or groups), through stripping during the ejection of extended components, or through the strict channelling of relativistic energy in beams which may supply the double sources[19]. Further work on the statistics of the ionised component, its kinematics and its spatial distribution would be of great interest.

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Search for X-ray emission from AO0235+164

THE BL Lacertae object, AO0235+164, is one of the best documented cases of the inverse-Compton scattering problem, that is, the brightness temperature of the emitting region, as implied by rapid variability and cosmological redshift distance, is well in excess of 10^{12} K. If the variable emission is produced by incoherent synchrotron radiation, then at these brightness temperatures the electrons would preferentially Compton scatter from their own synchrotron-emitted photons producing an excessive X-ray flux and subsequent cooling. In late-1975 AO0235+164 underwent an enormous outburst at radio[1,2], infrared and optical[3] wavelengths, for which Ledden *et al.*[2] deduced a brightness temperature $\geq 10^{15}$ K at 8 GHz. This source is also an active variable at low frequencies[4,5] (≤ 1 GHz). We describe here our search for X-ray emission from AO0235+164.

Explanations of this enormous outburst and related phenomena have invariably involved abandonment of one or more of the basic assumptions[6–9]: incoherent synchrotron emission by electrons, nonrelativistic expansion, cosmological redshift distance, or random geometry. Some of the explanations proffered, such as those involving coherent emission[7], synchrotron proton radiation[6] or special geometry[8], predict that the inferred brightness temperature is the observable brightness temperature of the varying region, although no such high brightness components ($\sim 10^{15-16}$ K) have been detected in recent searches for their expected interstellar scintillations[10–12]. In the case of AO0235+164, unfortunately, such a high brightness component may be unobservable due to possible angular broadening caused by the $z = 0.524$ absorption redshift system.

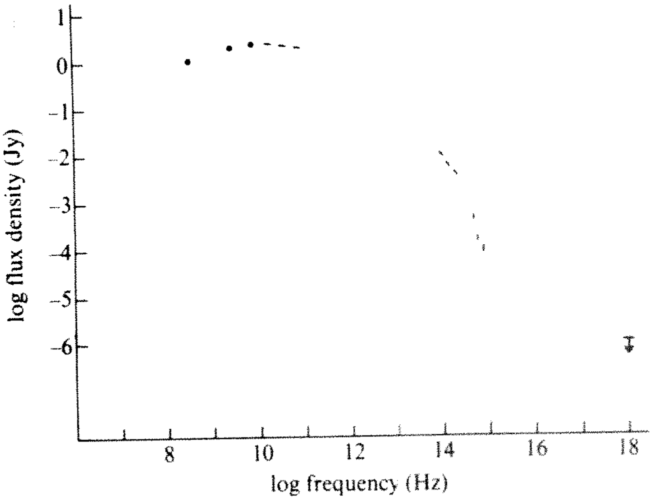

Fig. 1 Spectrum of AO0235+164. Vertical extent of bar (optical data) represents range of galactic extinction corrections used in ref. 21 (December 1977). This data is uncorrected for extinction in the $z = 0.524$ absorption redshift system. Dashed lines are based on observations at other epochs (see text). ●, Data from ref. 5 (March 1978); ⊥, this work.

It is clearly important to determine or set restrictive limits to the level of X-ray emission in compact objects. We chose AO0235+164 for our investigation on the basis of its previously discussed properties, and its high density of radiant energy inferred from VLBI observations[4]. Before its late-1975 outburst a limit of ~ 20 μJy at 10^{18} Hz could be set[13]. Since the outburst, the radio flux density has remained well above the 1974 minimum (0.6 Jy) indicating continuing injection of particles (Hugh Aller, personal communication).

The observations were carried out with the rotating modulation collimator (RMC) experiment aboard the SAS 3 X-ray observatory[14,15]. Both RMC systems having FWHM of 2.3 arc min and 4.5 arc min were used in the 2–6 and 6–11 keV bands. AO0235+164 was observed from 17.4 to 22.4 November 1977, a time when the Sun angle for this source was $\sim 180°$, thus maximising the usable data per orbit. The total exposure was 2.6×10^6 cm^2 s. Correlation distributions for different data channels and each orbit were constructed[16] and co-added to produce distributions for the entire observation in both energy bands. No emission was detected in either energy band at or near the best available radio position[17]. The limit to our sensitivity is imposed by counting statistics due to the background. The 3σ upper limit to the counting rate over the entire 2–11 keV band is 0.54 s^{-1}. This corresponds to a total flux limit of 2×10^{-11} ergs s^{-1} cm^{-2} over this range. The flux density at 10^{18} Hz is therefore ≤ 1.0 μJy. For the relatively flat X-ray spectrum expected for this source, these flux limits are insensitive to the assumed spectral index.

This upper limit allows us to place restrictions on first order Compton scattering, and therefore the brightness temperature and angular size, θ, of the compact radio source[18–20]. The overall spectrum around the time of our observation is shown in Fig. 1. The dashed lines indicate extensions of the radio and optical spectra into the infrared, strongly suggested by observations of this source at other epochs[21]. The optical flux may be depressed by several magnitudes of extinction in the $z = 0.524$ absorption redshift system[4], and thus the B-magnitude does not provide the

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best limit to first order Compton scattering. At the time of our observation the optical flux was near a minimum[21].

The radio spectrum does not exhibit a well-defined synchrotron self-absorption turnover. The very broad peak at 10 GHz could be best explained by an inhomogeneous synchrotron source[22], each component of which has a fairly flat spectral index. Ledden *et al.*[2] found $\alpha \approx 0.15$ in fitting the outburst spectrum to several self-absorbed components. Conservatively, we shall use $0.2 \leqslant \alpha \leqslant 0.5$, as large values of α produce the smallest lower limits on θ. Apparently, the radio flux decreases beyond 10 GHz. We shall, therefore, consider that the relevant component becomes optically thick at 10 GHz, as this also gives the smallest lower limit on θ. We also assume that the radio spectrum continues to at least 10^{12} Hz before cutting off, as suggested by an apparent continuity between the radio and infrared spectra[21]. This is necessary if Compton scattering is to produce X-rays at $\gamma^2 10^{12}\ \text{Hz} \approx 10^{19}$ Hz.

The maximum brightness temperature, T_n, occurring at frequency ν_n, is given by[20,23]

$$\frac{F^c_{\nu_c}}{F^s_{\nu_s}}\left(\frac{\nu_c}{\nu_s}\right)^{\alpha} \simeq \frac{16\pi}{3}\,\frac{j^{sc}_{\alpha 0}}{k_{\alpha 0}}\ln\Lambda\,\frac{r_e\nu_n}{c}\left(\frac{kT_n}{i_{\alpha 0}mc^2}\right)^{3+2\alpha}(1+z)^{4+2\alpha}$$

where $F^s_{\nu_s}$ is the synchrotron flux density at optically thin frequency ν_s, $F^c_{\nu_c}$ is the Compton-scattered flux density at frequency ν_c, $j^{sc}_{\alpha 0}$ and $k_{\alpha 0}$ are dimensionless functions of α (given in ref. 18), ln Λ is a function of the cutoffs in the electron spectrum[18], r_e is the classical electron radius, and m is the electron mass. We then find $T_n \leqslant 2.4 \times 10^{11}$ K, and correspondingly $\theta \geqslant 0.38$ m arc s, independent of distance (except through redshift corrections). This limit is derived for $\alpha = 0.5$. If, however, $\alpha = 0.2$, then we obtain $T_n \leqslant 1.5 \times 10^{11}$ K and $\theta \geqslant 0.50$ m arc s.

These values are to be compared with the 10.8 Ghz VLBI angular size measured in January 1978 with a transcontinental baseline (to be reported fully elsewhere by J. Broderick, *et al.*). It was found that $\geqslant$80% of the source is contained within a component of angular size $\leqslant$0.30 m arc s. In view of the various uncertainties involved in the application of the synchro-Compton model, and the interpretation of the VLBI visibility data, we do not consider this to be a major discrepancy. Of interest, however, is the possibility that it may indicate the presence of relativistic expansion in the source. Given the distance implied by the largest absorption redshift present (0.851) we can calculate the physical dimension of the source, l, and therefore the energy contained in relativistic particles, U_e, and magnetic field, U_B (refs 19, 23). Taking $\theta \simeq 0.30$ m arc s and $H_0 = 55$ km s^{-1} Mpc^{-1}, we find that $l \simeq 2.8$ pc, $U_e \simeq 3 \times 10^{54}$ erg, and $U_B \simeq 3 \times 10^{53}$ erg, for $\alpha \simeq 0.2$. If $\alpha \simeq 0.5$ then $U_e \simeq 1.4 \times 10^{55}$ erg and $U_B \simeq 5 \times 10^{52}$ erg. In either case the ratio of particle to magnetic energy, E_{em}, is large ($\geqslant$10), and the dynamics must be particle dominated. This would indicate relativistic expansion if the mean energy per particle is comparable to or greater than the rest energy of those particles that neutralise the electron plasma.

If the source is relatively local[6], then the physical size is reduced accordingly, the energy requirements are reduced substantially, but the dynamics remain particle dominated because $E_{em} \propto l^{-1}(1+z)^9$. The minimum of E_{em} occurs for $l \simeq 700$ Mpc, in which case $E_{em} \gtrsim 1.5$. Placing the source at a distance at which the brightness temperature inferred from the late-1975 outburst is 10^{12} K (~200 Mpc) gives $E_{em} \gtrsim 3$.

Wolfe *et al.*[4] found that the observed brightness temperature is $\gtrsim 10^{12}$ K at ~1 GHz, in excess of the inverse-Compton limit $\simeq 10^{12}\ \text{K}/(1+z) \simeq 5 \times 10^{11}$ K. In a static synchro-Compton model this would produce an excessive infrared or optical flux, further requiring that relativistic expansion be invoked.

The magnetic field, **B**, can be calculated independently of distance[19]. If $\theta \simeq 0.30$ m arc s, then $\mathbf{B} \simeq 0.4$–0.12 G depending on assumed spectral index. As long as $\mathbf{B} \leqslant 0.15$ G, then the electrons radiating at 10^{12} Hz are able to cross the source before losing their energy through synchrotron losses.

It would be interesting to determine better the magnitude of the discrepancy reported here. This can be accomplished by observations with the HEAO B X-ray observatory and VLBI observations over a longer baseline. Thus it will be possible to determine the extent to which relativistic expansion or other effects must be included in the synchrotron radiation models.

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IUE observations of the eclipsing binary Epsilon Aurigae

THE eclipsing binary Epsilon Aur is a most peculiar binary system and it has not been explained satisfactorily. We report here our observations of this system using the International Ultraviolet Explorer (IUE) collected at the Villafranca Satellite Tracking Station of the European Space Agency (SA).

Epsilon Aur is a single-spectrum binary whose visible component is a supergiant F0 Ia; it is eclipsed once every 27.1 yr by its invisible companion. The eclipse is total and lasts 714 d. The amplitude is 0.8 mag and is independent of wavelength. The eclipse is peculiar for these reasons: (1) during totality the spectrum of the primary F0 Ia star is always seen; (2) from the amplitude of the minimum the two stars might be expected to have about the same magnitude, a point which cannot be reconciled with the fact that only one spectrum is visible at maximum light, even when doubling of lines is expected. These inconsistencies can be removed by assuming that the invisible companion is surrounded by a disk or ring which is the eclipsing body. The fact that the minimum amplitude is independent of the wavelength was explained by assuming that the eclipsing body is a disk or ring of dust particles[1–3] or of ionised atoms

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which explain the opacity of the disk or ring as produced by electron scattering only[4].

This second hypothesis was suggested by the characteristics of the spectrum of the eclipsing body, which can be observed during the partial phases of the eclipse[4]. It is not identical to the F0 Ia spectrum, as would be expected if it were due to stellar light simply scattered by dust particles, but the lines originated from non-metastable levels are much fainter than those in the stellar spectrum which indicates that they are formed in a rarefied gas. However, the non-metastable levels of Mg I, Ca I and Fe I are more populated than is expected from the diluted radiation of the primary star at the surface of the eclipsing body. Hence, if the primary were responsible for the excitation of the eclipsing body (as suggested by Kuiper, Struve and Strömgren[5]), it must be richer than expected in UV radiation.

To explain the observations, it has been suggested[4] that the UV radiation does not come from the F0 primary, but from the invisible companion, which is assumed to be a hot star surrounded by a ring of ionised gas. Other suggestions[3,6] were that the secondary could be a black hole; the source of high-temperature radiation required for explaining the spectroscopic features of the eclipsing ring could be provided by the accretion mechanism, heating the gas at ~10^6 K (ref. 3).

To check these various hypotheses and discover the nature of the companion, we have observed the UV spectrum of Epsilon Aur with IUE, using the low-resolution mode (FWHM 5.5 to 7 Å) for the far UV (λλ 1150–1900 Å), and the high resolution mode (FWHM ~ 0.15 Å) for the near UV (λλ 1900–3200 Å). The observations were made on 19 April 1978 at phase 0.81 P. The exposure times were 30 min in the low-resolution, and 60 min in the high-resolution mode. The low-resolution spectrum indicates the presence of a hot object whose continuum is interrupted by several absorption lines and by a strong emission identified with λ 1302 O I (Fig. 1). The high-resolution spectrum is that expected for an F-type supergiant.

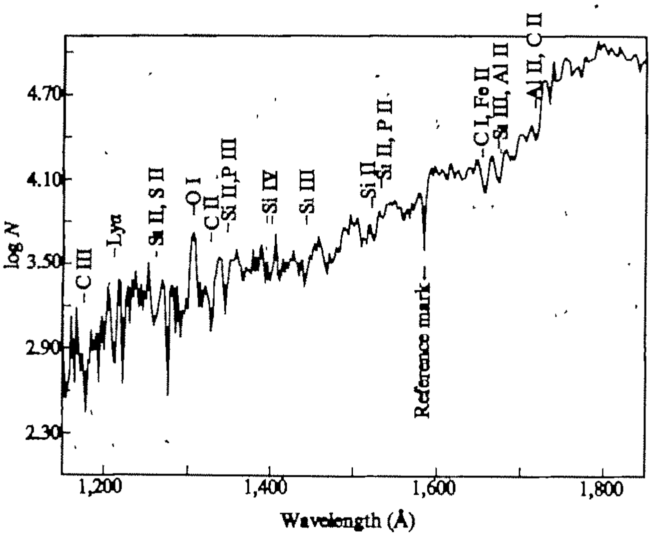

Fig. 1 The low-resolution short-wave spectrum of Epsilon Aur.

The output of the IUE software N, or net flux (corrected for the background), is transformed into absolute flux $\mathcal{F}(\lambda)$ (in erg cm^{-2} s^{-1} t^{-1}) by the relationship $\mathcal{F}(\lambda) = N(\lambda)S(\lambda)^{-1} t^{-1}$ where $S(\lambda)$ is the sensitivity of the IUE camera determined by Bohlin (personal communication) and t the exposure time (s). The values of the sensitivity should be considered as preliminary. Comparison of the fluxes from standard stars observed with OAO 2 and with experiment S2/68 on TD 1 with those observed with IUE (low-resolution mode, large slit 10″×20″) gives an uncertainty of 20% or better (as stated by the resident astronomers). However, our observations were made using the small aperture (3″ hole); in this case the light loss for both spectrographs is estimated to be ~50±20%. The transmission of the small aperture is grey to ±2%. Hence our measured flux is lower than the true flux but the energy distribution is not affected.

The continuum has been traced as a smooth line passing through the highest points of the whole spectrum N versus λ. The faint image at wavelengths shorter than the sensitivity limit at λ 1150 indicates the presence of light scattered from longer wavelengths. The amount of this scattered light is evaluated by measuring the net flux N remaining at the centre of Lyα ($N = 500$). By subtracting this value from the net flux we compute the absolute observed flux corrected for scattered light. Then the flux is corrected for interstellar extinction using the relationship of Nandy *et al.*[7] for $E(B-V) = 0.30$. This value of $E(B-V)$ is obtained by plotting the equivalent width of the interstellar absorption feature centred at λ 2200, $W = 144 \pm 30$ Å, on the relationship W (λ 2200) with $E(B-V)$ given by Nandy *et al.*[7].

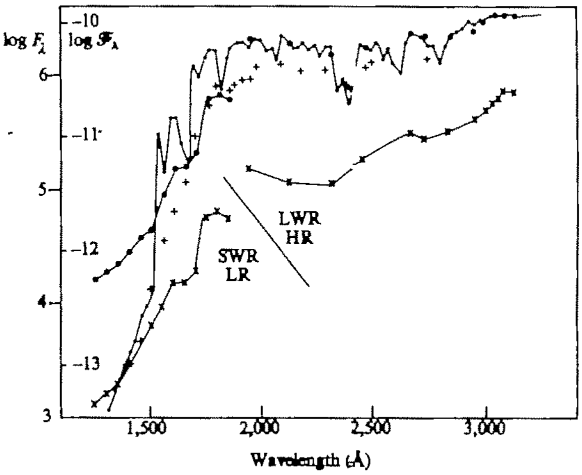

Fig. 2 The continuous flux $\mathcal{F}_\lambda$ (in erg cm^{-2} s^{-1} $Å^{-1}$) observed for Epsilon Aur (×, non-corrected for interstellar extinction, and ○, corrected for interstellar extinction with $E(B-V) = 0.30$), and that observed for Alpha Car with experiment S2/68 on TD 1 divided by 30(+) to make it comparable with that expected for Epsilon Aur which is 3.7 mag less bright than Alpha Car. F_λ is the surface flux computed for an atmosphere with parameters $T_e = 7{,}500$ K and $\log g = 1$ (●). SWP, LR, observations made in the low-resolution mode with the short wave primary camera; LWR, HR, observations made in the high-resolution mode, with the long wave redundant camera.

The continuous flux corrected for scattered light and for interstellar extinction is compared with that observed for Alpha Car (F0 Ia, $E(B-V) = 0.01$) with experiment S2/68 on TD 1 (ref. 8) and with the theoretical surface flux computed by Kurucz et al.[9] for $T_e = 7{,}500$ K and $\log g = 1$, solar composition (a model which reproduces the visual spectrum well[10]) (Fig. 2).

The ratio

$$r_\lambda = \frac{\mathcal{F}_{\lambda\,\mathrm{obs}}}{\mathcal{F}_{\lambda\,\mathrm{pred}}} = \frac{R_A^2 F_{\lambda A} + R_B^2 F_{\lambda B}}{R_A^2 F_{\lambda A}}$$

(where $\mathcal{F}_{\lambda\,\mathrm{obs}}$ is the observed flux at Earth corrected for interstellar extinction, and $\mathcal{F}_{\lambda\,\mathrm{pred}}$ is the predicted flux at Earth computed assuming that Epsilon Aur has the same energy distribution as Alpha Car; $F_{\lambda A}$ is the flux emitted by a stellar atmosphere with $T_e = 7{,}500$ K, $\log g = 1$ computed by Kurucz *et al.*[9]; $R_A = 10^{13}$ cm is the radius of the primary as indicated by the circumstances of the eclipse) has the following values:

$r(\lambda\ 1300) = 50 \pm 30$

$r(\lambda\lambda\ 1400\text{–}1500) = 8.1 \pm 0.4$

$r(\lambda\lambda\ 1550\text{–}1600) = 2.3 \pm 0.3$

$r(\lambda\lambda\ 1650\text{–}3100) = 1.1 \pm 0.3$

From these data we can estimate that the effective temperature and the radius of the companion have the following values:

$$T_B = 15{,}000 \pm 3{,}000\ \text{K}, \quad R_B = 10^{11} \pm 0.5 \times 10^{11}\ \text{cm}$$

The absorption line spectrum confirms that the companion is not very hot: λ 1640 He II and λλ 1548–1550 C IV are not present, while λλ 1394–1402 Si IV are. All the strongest lines of C I, C II, C III, N I, O I (in emission), Si II, Si III, Si IV, P II, P III, S II, S III, and of once- and twice-ionised atoms of the iron group are present.

The strong emission of O I at λ 1302 indicates the existence of an envelope where the hydrogen is mostly ionised, because H I and O I have almost identical ionisation potentials. Lyβ emission can overpopulate the upper level of λ 1025.77 O I, which has almost the same high EP as Lyβ; then by cascade, emissions at λ 8446 and λ 1302 can be explained[11,12].

The companion is therefore a B-type star falling on or slightly below the main sequence, whose absolute bolometric magnitude is about 7 mag fainter than that of the primary. As the system is not close, the presence of the ring cannot be explained by mass exchange between the two stars. The companion may be a past nova, and the ring may have formed with gas expelled during an outburst. Absolute magnitude and spectral type of quiescent recurrent novae have values similar to those derived for Epsilon Aur B.

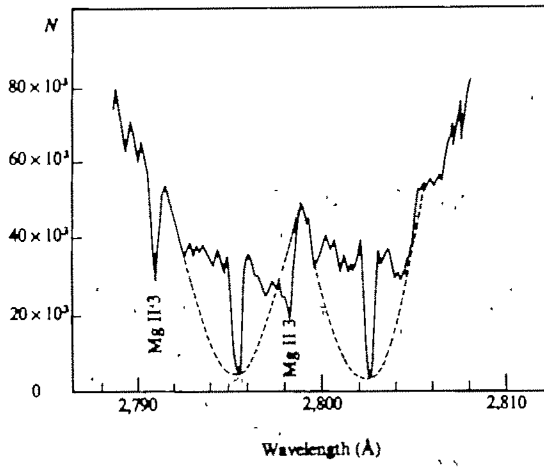

Fig. 3 The profiles of the Mg II resonance lines and the Mg II lines of multiplet 3. The ordinates are IUE counts, corrected for the background. Continuum $N = 130 \times 10^3$.

The near UV spectrum is characterised by strong absorption lines of once-ionised metallic atoms. The most interesting features are the Mg II resonance lines, which have already been observed with BUSS in 1976 but were strongly under-exposed. In the present spectrum (Fig. 3) it is evident that the central part is filled with emission, and this is cut by a strong absorption core which can be formed in the chromosphere of the F0 supergiant or be an interstellar feature, or a blend of the two. From the intensity of the Mg II interstellar lines versus distance relationship given by Lamers and Snijders[13] it seems probable that they are interstellar features. The profile of the Mg II resonance lines is very similar to that observed for Alpha Car[14], but the width of the emissions is 5.1 Å and that of Alpha Car 1.9 Å, suggesting that Epsilon Aur has a larger luminosity than Alpha Car. By plotting the width of the emission w (in km s^{-1}) on the relationship M_v against log w, given by Evans *et al.*[14], a value $M_v = -7$ is found for Epsilon Aur. The flux in the emission is about 2.5×10^{-12} erg cm^{-2} s^{-1} Å^{-1}, and after correction for interstellar extinction is 1.4×10^{-11}. The analogous flux for Alpha Car is 3.1×10^{-10}, that is 22 times that for Epsilon Aur, while the expected value due only to their difference in apparent magnitude is 30 times that of Epsilon Aur.

We thank all the staff of VILSPA Observatory for their assistance in observing and reducing the data, Dr M. Penston for calling our attention to the existence of scattered light in the far UV spectrum, and Dr P. Santin for assistance in data processing with the DEC PDP-11/45 computer system of Trieste Observatory. This work was supported by a CNR-SAS contract.

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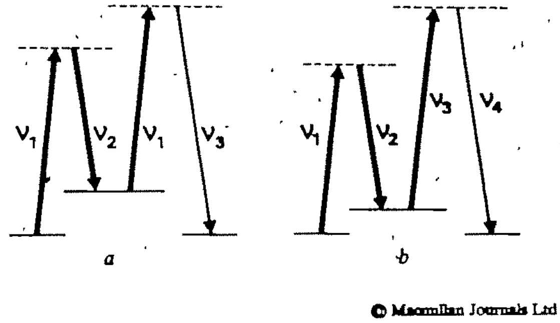
Low frequency coherent anti-Stokes Raman spectroscopy of air

THE techniques of coherent Raman spectroscopy are revolutionising the applications and scope of Raman spectroscopy. Particularly important aspects are: very high resolution spectroscopy (the Doppler width being proportional to the Raman shift of the band under study); nanosecond (and sub-nanosecond) spectroscopy, where the resolution obtained may be limited by the Fourier transform of the experimental time scale; discrimination against luminescence from the sample (whether laser induced or not). We discuss here coherent anti-Stokes Raman spectroscopy (CARS) at low frequency shifts.

The technique is briefly as follows: when a sample is irradiated with two lasers at frequencies ν_1 and ν_2 a signal is generated at a frequency $2\nu_1 - \nu_2 = \nu_3$ (see Fig. 1*a*). The signal intensity is proportional to $I^2_{\nu_1} \times I_{\nu_2}$ (where I is the incident beam intensity). When $\nu_1 - \nu_2$ is tuned to be equal to a molecular Raman active transition frequency resonance occurs. Furthermore, the signal at ν_3 is emitted to be a diffraction limited beam, in ideal phase matching conditions. There have been many reports on the use

Fig. 1 Schematic representation of *a*, three-colour CARS; *b*, four-colour CARS.



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of CARS, notably on gases such as N_2, C_2H_2 (ref. 1) and D_2 (ref. 2). However, we know of only one low-frequency spectrum[3] (equivalent to a Raman shift of ~100 cm^{-1}). In that example the scattering medium was a liquid, while the technique we describe here is suitable for gases at pressures appreciably below atmospheric. Because CARS signals scale normally as the square of the number density, this represents an improvement in sensitivity of more than four orders of magnitude.

The lasers used for our initial experiments were:

Fixed frequency ν_1: Flash lamp pumped Nd/YAG frequency doubled to 532 nm (JK Lasers)
Tunable frequency ν_2: Flash lamp pumped dye laser (Chromatix CMX-4).

Using the above equipment, but with the Nd/YAG laser in the unstable resonator configuration, CARS spectroscopy on gases using colinear beams is routine in the range 1,000–3,200 cm^{-1}. Below 1,000 cm^{-1} problems arise due to resonant CARS signals from optical components notably in lenses and windows (where the number density is high). The CARS signal from the glass can be removed conveniently by beam expansion before beam combination and passage through the lenses and windows. Where the cell windows have to be close to the beam waist, CARS signals may be greatly reduced in one of two ways. Either the window material is selected to have no first order Raman spectrum and contains atoms of low polarisability (such as LiF) or the windows are very thin. In this way spectra may be run relatively routinely to 600 cm^{-1} for moderately scattering gases at tens of torr pressure.

Below 600 cm^{-1} the major problem lies in the collimated broad band superradiance emitted at the signal frequency by the tunable dye laser. An additional difficulty is the separation of the weak (wanted) signal from the other two intense (unwanted) colinear beams. Both of these problems become increasingly important as one moves to smaller and smaller Raman shifts.

An alternative experiment uses two tunable dye lasers (ν_1, ν_2) and one fixed frequency laser (ν_3) which is considerably removed in frequency from both the dye lasers, as shown in Fig. 1*b*. Providing ν_1 and ν_2 are well removed from ν_3 in frequency, the dye laser beams can be readily separated from the fixed frequency laser ν_3 with its associated CARS signal ν_4. Superradiance problems with fixed frequency solid state lasers are negligible compared with those from dye lasers.

This arrangement was realised experimentally with a purpose built step-tunable dye laser (ν_1) pumped by 80% of the frequency doubled Nd/YAG output. The remaining 20% of this 532 nm radiation was used as the fixed frequency (ν_3), while the Chromatix CMX-4 acted as the tunable laser (ν_2). In this way, ν_3 is particularly free from other frequencies due to the square law dependence (on the intensity of the incident beam) for the frequency doubling process.

The remaining difficulty is the separation of ν_3 from the signal. This may be achieved by using prisms or gratings of sufficient dispersion. However, for pure rotational Raman spectroscopy all bands are depolarised. In three-colour CARS (Fig. 1*a*) the polarisation of the CARS radiation follows that of ν_2. The situation is more complex in four-colour CARS[4] (Fig. 1*b*). For depolarised bands (or polarised bands where the anisotropy is large) polarisation of the CARS signal (ν_4) will be exactly perpendicular to ν_3 if the polarisations of ν_1 and ν_2 are orthogonal and ν_3 is parallel to either ν_1 or ν_2. By passing all four beams through an analysing polariser and a prism of moderate dispersion, ν_1 and ν_2 can be effectively removed and ν_3 attenuated by six orders of magnitude. The limitation in the removal of ν_3 is the birefringence of the optical components. Additional filtering of the signal with a conventional double monochromator allows the monitoring of ν_4 to approximately a shift of 40 cm^{-1}.

The success of this approach is clearly demonstrated by the pure rotational CARS spectrum of air in Fig. 2. The inset shows a higher resolution spectrum of the 60 cm^{-1} band arising from a near coincidence (~0.7 cm^{-1}) of $^{14}N_2$ $J = 6 \rightarrow 8$ and $^{16}O_2$ $J = 9 \rightarrow 11$. The resolution of 0.2 cm^{-1} is routine and 0.01 cm^{-1} could readily be achieved, although not using the CMX-4.

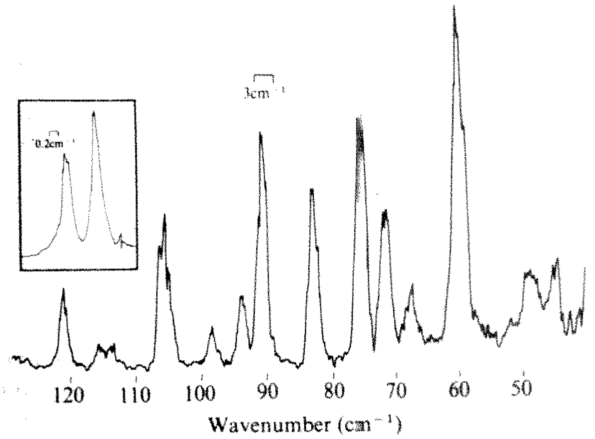

Fig. 2 Pure rotational CARS spectrum of air. Inset: the 60 cm^{-1} band under higher resolution.

For strongly polarised bands the BOXCARS approach of Eckbreth[5], using non colinear beams coupled with the present four colour system seems to be attractive.

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Trace elements in diamonds of different types

STUDIES of optical and electrical properties have shown that natural diamond can be broadly categorised into four types, types IA, IB, IIA and IIB (refs 1–3). Type IA is the most common (98% abundance)[3] and contains up to 0.34% N (ref. 16), which has been argued[5] to occur in atom pairs. Type IB diamond contains much less N, of which 1–2% occurs as single substitutional paramagnetic N. Type II diamonds contain even less N. The p-type semiconducting property of type IIB diamond is due to the presence of B (refs 6, 7), the concentration of which has been correlated with the acceptor concentration[7]. We have determined and report here the impurity contents of 23 diamonds of different types in order to investigate whether elements other than N and B contribute to the physical properties of diamond. Although there are few diamonds of each type and the statistics are therefore poor, two diamonds were found to contain an excess of Ni, while the three Type IIB diamonds possibly contained an excess of Al.

The diamonds analysed were classified into the different types using infrared and ultraviolet spectral characteristics, and

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conductivity measurements. Diamonds 1 to 6 (Table 1) were flawless light yellow octahedra. In all the other diamonds inclusions and flaws were visible at 50× magnification under polarised light and these were removed by cutting and polishing. The individual diamonds were analysed using an established neutron activation analysis technique[4,8]. Superficial contamination was avoided by chemical etching after irradiation to remove a few micrometres of diamond from the outer surface[9]. The results are given in Table 1.

Errors vary with sample size and impurity concentration, and range from 5% at best to 40% at the limit of detection. The most reliable data are for Na, Al, Sc, Mn, Co and Ni. Because of the interest in Ni, limits of detection were calculated for this element[10]. Rare earth elements (REEs) were observed in some diamonds at very low concentrations, but no reliable quantitative determinations could be made.

Despite the care taken to remove inclusions it is clear from the trace element concentrations and their inter-relationships[8,11] that diamonds nos 4, 8, 9, 10, 18 and 20 contained mineral inclusions (Table 1). The impurities present in the rest of the diamonds were inferred to be predominantly due to submicroscopic inclusions of the parental magma[11].

Considering, for example, the relationship between Al and Mn for the 23 diamonds studied (Fig. 1), and ignoring the inclusion-containing stones, there seems to be no significant difference between types IA and IIA diamonds. This observation is supported by more detailed scrutiny of the data in Table 1, and it seems that the present data on trace element levels can contribute little to the existing body of knowledge which attributes the difference between types IA and IIA diamond to the N content, and the physical mode of its occurrence.

The data in Table 1 show that among the inclusion-free diamonds the type IIB diamonds contained the highest levels of Al. A plot of the Al against Mn contents (Fig. 1) shows that the Al content is in excess of that expected from the submicroscopic inclusions of parental magna inferred to be present in all diamonds[11]. That this is an Al excess and not a Mn deficiency can be shown by comparing the Al with other lithophile elements, for example, the Al to Sc ratio is ~3,000 in the parental magma (H.W.F. unpublished data). It is difficult to determine the real significance of the Al excess in type IIB diamonds because comparable Al excesses are occasionally found in other types of diamond, for example, nos 2, 3 and 7 (Table 1). There has been controversy over the part played by Al in semiconducting diamond[12], and it has been suggested that Al

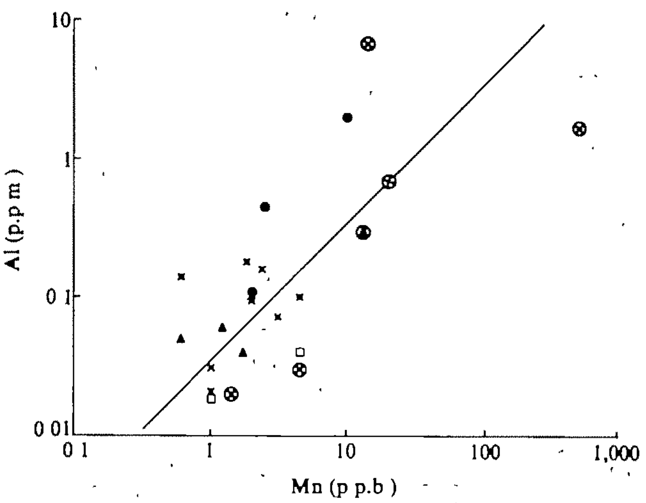

Fig. 1 Aluminium and manganese content in diamonds of different types: ×, type IA; □, type IA; △, type IIIA; ●, type IIB. Points circled are from diamonds containing inferred mineral inclusions. The composition line shows the Al:Mn ratio of ~35 inferred by Fesq et al.[11] for the parental magma (p.p.b., parts per 10^9).

Table 1 Trace element concentrations in typed stones

Diamond	Mass (mg)	Na (p p.b.)*	Mg (p.p m.)	Al (p.p.m.)	K (p.p.m.)	Sc (p.p.b.)*	Ti (p.p.m.)	V (p.p.b.)*	Cr (p.p.b.)*	Mn (p.p.b.)*	Fe (p.p.m.)	Co (p.p.b.)*	Ni (p.p m.)	As (p.p.b.)*
Type IA														
1	76	0.1	—	—	—	0.01	—	—	—	0.2	1.0	2.3	0.07	0.01
2	68	0.1	—	0.14	—	0.02	0.05	—	—	0.6	0 3	0.4	0.01	—
3	57	—	—	0.16	—	0.02	0.1	—	—	2.4	—	0.3	<0.01	—
4†	54	1.0	—	0.02	—	—	—	0.6	—	1:5	0.3	4.5	0.07	—
5	59	1.0	—	0.03	—	0.02	—	0.7	—	1.0	—	0.3	<0.01	0.02
6	38	—	—	0.02	—	0.04	0.05	—	—	1.0	0.2	0.3	<0.012	—
7	98	0.1	—	0.18	—	0.01	—	—	0.5	1.8	—	1.0	<0.015	—
8†	40	2900	8.0	6.9	0.17	0.6	—	20	110	14	1.3	3.0	0.08	—
9†	32	260	200	1.7	0.07	2.2	—	15	950	500	47	200	7 0	—
10†	38	190	0.6	0.03	0.03	0.04	—	0.8	2 0	4.5	1 0	0.7	<0.012	—
16	25	6.0	—	0.10	—	0.02	—	1.2	—	4.3	0.2	0.6	<0.015	—
17	27	0.4	—	0.1	—	0.04	—	—	—	2.0	0.3	0.4	<0.015	—
18†	80	0.8	—	0.7	—	—	0.25	3.2	90	20	30	340	40	0.6
19	16	—	—	0.07	—	0.08	—	—	—	3.0	0.2	0.7	<0.03	0.03
20†	28	35	6.0	0.3	0.01	0.03	—	—	2.0	13	0.6	2.0	0.07	—
Type IB														
11	132	0 2	—	0.02	—	0.01	—	—	—	1.0	<0.05	0.4	1.0	—
12	50	0.1	—	0.04	—	0.03	—	—	—	4.5	<0 1	0.5	0.7	—
Type IIA														
13	128	2.0	—	0.04	—	0.01	—	—	0.8	1.7	—	0.1	<0.005	—
14	42	—	—	0.06	—	0.03	—	—	—	1.2	0.1	0.3	<0.016	—
15	116	0.6	—	0.05	—	0 01	—	0.3	—	0.6	—	0.1	<0.004	0.01
Type IIB														
21	105	1.0	1.0	2.0	—	0 1	0.25	—	1.0	10	0.1	0.4	<0.006	0.01
22	44	1 0	—	0.44	—	0.03	0.35	—	—	2.5	0.1	0.3	<0.012	—
23	107	0.2	—	0.1	—	0.02	0.08	—	—	2.0	—	0.1	<0.004	—

Some elements were determined in only a few of the diamonds. Diamond no. 8 contained 20 p.p.m. Ca, Diamonds nos 9 and 18 contained 0.5 and 1.1 p.p.m. Cu, respectively. REE's were observed in diamonds nos 4, 8, 10, 18 and 20.

*Parts per 10^9.

†Diamonds with inferred mineral inclusions.

could act as a getter for N (ref. 13), thus preventing compensation of B. A larger number of type IIB diamonds is being analysed for B, N and Al in order to clarify any secondary role which may be played by Al in semiconducting diamonds.

As Table 1 shows, two diamonds with types IB character (Nos 11 and 12) have unusually large Ni:Co ratios (2,500 and 1,400) compared to all other diamonds. With the exception of these two diamonds, the Ni:Co ratios in the diamonds analysed here ranged from <15 to 118. This range includes the ratios for those diamonds where Ni was not detected but was assumed to be present at the limit of detection. This range is in good agreement with that found previously where Ni:Co ratios ranging from 5 to 110 were found in a large suite of diamonds[4] (note error in ref. 11, Table 3). Those diamonds were not typed but probably contained predominantly type IA diamonds, the most common type. One exception found in that work was for a 1 g sample containing about 15 orange diamonds from the Premier Mine, Transvaal. This sample, which probably contained some type IB diamonds, had a Ni:Co ratio of 250, which again is unusually high. These ratios should be compared to those inferred for the parental magma: silicate phase ~15; sulphide phase ~40 (H.W.F. unpublished data).

In this case it is more difficult to prove which of the two elements is anomalous. However, it is unusual for the Fe:Ni ratio to be <1. In the data obtained from the analysis of over 100 samples of diamond only nine cases were found where the Fe:Ni ratio was <1, the lowest being 0.14. From the calculated limits of detection for iron in diamonds nos 11 and 12, the maximum possible ratios are seen to be 0.05 and 0.14, respectively, which are unusually low[4]. Because both the Fe and the Co contents are low compared to that of Ni in diamonds nos 11 and 12 only, it suggests that these diamonds are characterised by a net Ni excess and are therefore unusual.

Two arguments can explain the high Ni:Co ratio found in these two diamonds. First, it could be due to submicroscopic inclusions of a Ni-rich, Co-poor, phase. The low levels of lithophile elements (for example, Al and Sc) found in these two diamonds suggests that any possible Ni-rich phase should be a sulphide. Alternatively, it has been shown[14] that Ni can occupy lattice sites in synthetic diamonds grown from Ni-rich melts, where Ni acts as a catalyst. Klyuev *et al.*[15] regard the morphology of type IB diamond, and the presence of paramagnetic nitrogen, as indicating formation in a low-temperature environment. If this is so then the availability of Ni as a catalyst could be essential to their formation. Unfortunately the two Ni-rich stones cannot be unambiguously classified: diamond no. 12 is dominantly type IB with some admixture, while diamond no. 11 is most likely mixed type IB and type IIA.

These speculations reflect the need for further measurement of the Ni:Co ratios in type IB diamonds. High purity diamonds are necessary as the presence of inclusions with a 'normal' silicate or sulphide composition will tend to mask any unusual Ni:Co ratio. A search for Ni in the lattice of type IB diamonds using other methods of analysis could help clarify the possible role of Ni in the formation of type IB diamonds.

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A reduction in $^{87}Sr/^{86}Sr$ during basalt alteration

HYDROTHERMAL alteration of basalts on the ocean floor has been reported to cause a general increase in the $^{87}Sr/^{86}Sr$ ratio of the basalts[1,2]. Results are presented here from Mull in the British Tertiary province which suggest strongly that hydrothermal alteration in such discrete magmatic centres may actually cause a reduction in the $^{87}Sr/^{86}Sr$ ratios of some basalts. Preliminary data indicate that the $^{143}Nd/^{144}Nd$ ratios are unaffected.

Oxygen isotope studies[3,4] of Tertiary igneous rocks from Skye, Mull and Ardnamurchan have demonstrated that large scale hydrothermal systems were established in these areas at the time of igneous intrusion. On both Skye and Mull hydrothermal aureoles extend for up to 6 km from the central intrusive complexes into the surrounding basalts. Within these regions greenschist-facies mineral assemblages have been formed and the rocks are depleted in ^{18}O due to interaction with hot meteoric waters.

The samples selected for this study are from a quarry 3.5 km east of Salen, which lies within the epidote zone of the central aureole on Mull. A hydrothermally altered lava flow is exposed which shows a gradual transition from dark basalt in the massive flow interior to green 'spilitic' material at the margin. In the centre of the flow only olivine has broken down; it is pseudomorphed by chlorite and oxides and the groundmass is rich in chlorite. With increasing alteration plagioclase and the titanomagnetites are replaced by albite, sphene, and calcite and/or epidote. Only at the margin of the flow is pyroxene completely broken down (Fig. 1) and an assemblage of chlorite, albite, quartz, amphibole, epidote, and sphene occurs in which none of the original mineralogy is preserved. Within this flow Al, Ti, Zr, Nb, Y, P, and the rare earth elements preserve almost constant ratios except at the margin where slight loss of *P* and the light rare earths (La, Ce) is observed[5–7]. This is accompanied by variations in the other major and trace elements; particularly, drastic leaching of Ca. Sr isotope analyses were carried out on 10 whole rock samples across the lava flow and on leached pyroxene separated from the flow centre. A secondary mineral pod consisting of zeolite, calcite, and albite surrounded by chlorite which formed immediately above the base of the overlying lava flow, and two discordant veins were also analysed to determine the $^{87}Sr/^{86}Sr$ ratio of at least some of the hydrothermal fluids.

The results are presented in Table 1. Within the lava flow the age-corrected initial $^{87}Sr/^{86}Sr$ ratios range from 0.70467 near the top of the flow to 0.70519 at the base of the sampled section (Fig. 1). The decrease in $^{87}Sr/^{86}Sr$ towards the top of the flow is accompanied by an increase in the degree of visible alteration and a decrease in Fe^{3+}/Fe^{2+}. Sr contents range between 270 and 440 p.p.m. in the more altered samples but seem to be much less variable near the centre of the flow.

$^{143}Nd/^{144}Nd$ ratios were determined on two samples using the technique described in ref. 8. The two samples show very

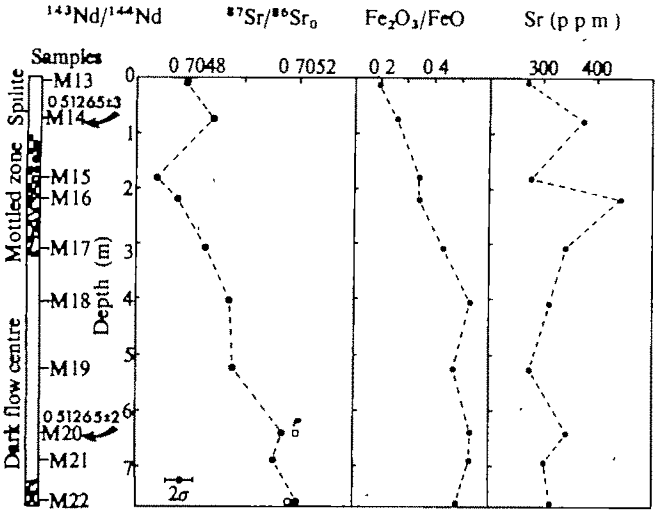

Fig. 1 Variations in ^{87}Sr/^{86}Sr$_0$, Fe$_2$O$_3$/FeO, and Sr p.p m across 7.5 m of an individual lava flow, near Salen, Mull. The degree of hydrothermal alteration is illustrated and M22 shows the onset of increased alteration approaching the base of the flow. Open symbols denote samples leached in 6M HCl; □, pyroxene; ○, whole rock.

different degrees of alteration but in sharp contrast to their variable ^{87}Sr/^{86}Sr ratios (Fig. 1) their Nd compositions are indistinguishable analytically (M14, 0.51265±3; M20, 0.51265±2; 2σ errors).

To investigate further the Sr isotope geochemistry, pyroxene was separated from M20 and it and some whole rock powders were subjected to leaching experiments. Leaching was carried out with 6M HCl in sealed Teflon bombs for 14 h at 130°C (ref. 9). X-ray analysis of the whole rock powders before and after leaching showed a marked increase in the concentration of pyroxene and to a lesser extent plagioclase, due to the dissolution of secondary minerals such as chlorite. Analysis of the residue after leaching should therefore provide ^{87}Sr/^{86}Sr ratios close to the pre-alteration composition of the lava flow.

The pyroxene has an age-corrected initial ^{87}Sr/^{86}Sr ratio of 0.70519 while that of the leached whole rock sample M22 is 0.70516 (Fig. 1). Rb and Sr concentrations for the leached residues from M18, M19, and M20 are not available but they all have significantly higher present-day ^{87}Sr/^{86}Sr ratios than the unleached samples. This indicates that the altered material tends to have lower ^{87}Sr/^{86}Sr ratios than the fresh basalts.

The leaching experiments and the observed decrease of ^{87}Sr/^{86}Sr with increasing bulk rock alteration and decreasing Fe^{3+}/Fe^{2+} (Fig. 1), all suggest that hydrothermal alteration of this lava flow resulted in a general reduction in ^{87}Sr/^{86}Sr. The composition of the separated leached pyroxene provides the best estimate of the primary composition of the magma (~0.7052). The two ^{143}Nd/^{144}Nd results, however, indicate that the lava was isotopically homogeneous when it crystallised, and that Nd was not affected significantly by the hydrothermal alteration (see also ref. 7).

Evidence that the fluids responsible for the hydrothermal alteration did have low ^{87}Sr/^{86}Sr ratios is provided by the results on secondary minerals (Table 1). The secondary pod from the overlying flow gave values of 0.70421 and 0.70427. The two vein samples M64 and M24 which cut the flow and therefore represent later stage fluids have ^{87}Sr/^{86}Sr ratios of 0.70467 and 0.70521. This may also indicate a rise in the value of the ^{87}Sr/^{86}Sr ratio of the fluids with time at this locality.

Forester and Taylor[4] have suggested that the alteration within the hydrothermal aureole on Mull was caused by interaction with fluids having δ^{18}O values of about −11 to −12. Such depleted oxygen compositions are suggestive of meteoric water in high latitudes, but at least in the area of this study the fluids are also apparently characterised by low ^{87}Sr/^{86}Sr ratios (< 0.7048). This clearly rules out interaction with Moinian basement[10], or Tertiary intrusives such as the Glen Cannel and Loch Usig granophyres which have initial ^{87}Sr/^{86}Sr ratios of 0.7094±3 and 0.7135–0.7162 (refs 11 and 10) respectively. Sources of the unradiogenic Sr in the fluids are either unconfirmed but possible granulite facies basement, or, much more likely, the basalts themselves.

We envisage that interaction of meteoric water with hot volcanic rocks caused the chemistry of the resultant fluids to be buffered by that of the basalts. As 70% of the fresh volcanic rocks from Mull analysed by Beckinsale *et al.*[11] have ^{87}Sr/^{86}Sr ratios <0.7043, the fluids in this area are likely to have similar ^{87}Sr/^{86}Sr ratios. Their interaction with basalt of Sr composition 0.7052 will therefore result in a reduction in ^{87}Sr/^{86}Sr in the

Table 1 Rb–Sr results

Sample	Lithology/mineralogy	Rb*	Sr*	Rb/Sr	^{87}Sr/^{86}Sr†	(^{87}Sr/^{86}Sr)$_0$‡
M13	Altered basalt, chl–plag–qtz–sph	7.17	271	0.026	0.70486±3	0.70479
M14	Altered basalt, some relict cpx	1.76	375	0.005	0.70489±3	0.70488
M15	Altered basalt, increasing growth of	2.51	279	0.009	0.70469±3	0.70467
M16	secondary minerals 16–19% relict cpx	11.8	443	0.027	0.70482±3	0.70475
M17	Similar to M15, M16	14.5	338	0.043	0.70496±3	0.70485
M18	Less altered basalt; mainly chl pseudomorphs after ol	15.4	310	0.050	0.70506±4 (0.70519±2)	0.70494
M19	Some growth of sphene and albite	11.6	274	0 042	0.70505±5 (0.70514±4)	0.70495
M20	Similar to M18	2.62	340	0.008	0.70515±2 (0.70525±6)	0.70513
M20	Pyroxene	(0.26)	(51 9)	(0.005	(0 70520±2)	(0.70519)
M21	Similar to M18	9.08	299	0 030	0.70519±3	0.70511
M22	Similar to M15, M16	14.6 (11 6)	311 (129)	0.047 (0.090)	0.70531±3 (0.70538±4)	0.70519 (0.70516)
M24	Calcite and laumontite vein	2*	169*	0.012	0.70521±6	0.70519
M64	Laumontite vein	2*	359*	0.006	0.70467±3	0.70466
M65	Altered basalt adjacent to M64				0.70505±4	
M75A	Alteration pod: chl–laumontite–cc				0 70421±6	
M75B	Alteration pod: cc, laumontite				0.70427±4	

Rb and Sr contents determined by isotope dilution (±1%) unless postscripted* which denotes XRF analysis chl, chlorite; plag, plagioclase; qtz, quartz; sph, shene; cpx, clinopyroxene; cc, calcerous; ol, olivine.

† present day ^{87}Sr/^{86}Sr ratio ±2 s.e.m. E–A = 0.70807±3 and NBS 987 = 0.71029±3 during this study.

‡ Initial Sr composition calculated assuming an age of 60 Myr and $\lambda = 1.39\times10^{-11}$ yr^{-1} for ^{87}Rb decay.

Results in brackets indicate that they were determined on samples which had been leached in 6M HCp(see text) more altered material (see Fig. 1). Moreover as no systematic variation in Sr content in the altered rocks was observed (Fig. 1) it seems that Sr isotope exchange took place without net addition or removal of Sr. Finally these results may provide some constraints on our models for the size of water circulation 'cells'. They suggest that the Sr composition of the fluids in this area was not affected significantly by interaction with either 'old' basement (perhaps 0.5 km beneath the present erosion surface) or Tertiary intrusive rocks which crop out only 1.5 km to the south.

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Field measurements of the rheology of lava

KNOWLEDGE of magma rheology is required to understand many igneous and volcanic processes. Viscosities of silicate melts can be estimated from their chemical composition and temperature using empirical methods[1-3]. However, the validity of these estimates has been established only at supraliquidus temperatures where common igneous melts behave as newtonian fluids. Recent experimental evidence from field and laboratory studies[4-7], has demonstrated that, at subliquidus temperatures, some magmas behave as non-newtonian fluids due to the presence of dispersed crystals and gas bubbles and possibly as a consequence of structuring in the silicate melt. We present data here on the rheological properties of small lava flows which were erupted on Mount Etna in 1975. These data were obtained by field measurements using a new field viscometer[8], a shear vane attached to a torque wrench, a conventional penetrometer and various field techniques for estimating rheological properties from the dimensions of lava flows.

Published field measurements of lava rheology have been obtained using various techniques[4,9-12] which, with the exception of one, assume newtonian rheology and also fail to take account of marked thermal (and therefore rheological) gradients which develop at the margins of active flows. The most accurate lava rheology data to be published[4] indicates that the tholeiite in the Makaopuhi lava lake in 1968 was pseudoplastic with yield strengths of 70 and 120 N m^{-2} measured in two experiments and plastic viscosities of 750 and 650 Pa s respectively. The rotating shear vane used for these measurements, however, is suitable only for stable encrusted lava lakes. Because moving lava flows are generally encountered during volcanic eruptions, alternative methods of measuring lava rheology are required. We describe here several techniques with different levels of sophistication which can be used to measure rheological properties of active lava flows.

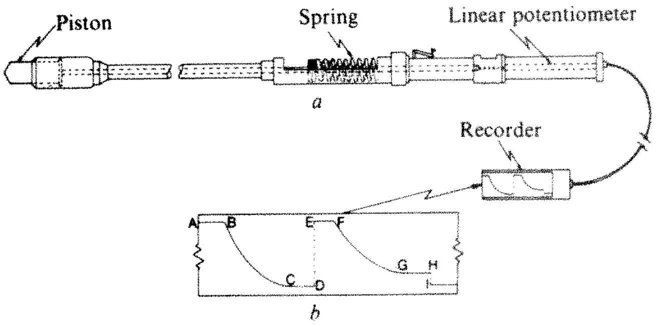

Fig. 1 The Mark 2 viscometer and recorder are shown in (*a*) while an enlarged view of a typical trace from the recorder is shown in (*b*). The Mark 2 is 2 m long and is constructed from high temperature stainless steel. The stainless steel spring used on Etna had a strength of 0.155 N m^{-1}. The recorder, a Cambridge type 72125 single speed hot wire recorder, had a chart speed of 0.0245 m s^{-1}. The traces in (*b*) are characteristic of a newtonian fluid (A–D) and a Bingham material (E–H). For a newtonian fluid, the horizontal distance between A and D is a function of newtonian viscosity. For the Bingham material, GH corresponds to the equilibrium position of the piston in the material; thus the vertical distance HI is related to the dynamic yield strength of the material.

The Mark 2 field viscometer (Fig. 1) is essentially a penetrometer, but differs from conventional penetrometers in that shearing is confined mainly to the region adjacent to the advancing piston in the interior of the lava. Shearing resistance is not affected by the more viscous cool flow margins as with a conventional penetrometer. In the field the instrument, which is made from BS 321 S20 stainless steel, is preheated by repeated insertions into lava until the temperature of the 'active' end is comparable to that of the lava. It is then inserted to the required depth in the lava and the piston released. A controlled but variable shear rate around the advancing piston is obtained using a compressed spring as the propelling force (Fig. 1) and piston velocity, at a given axial force, is monitored by a linear potentiometer on a hot wire pen recorder. The recorder output is readily converted to shear stress–shear rate data[8]. The yield strength of the lava is obtained directly from the residual force applied by the piston at zero piston velocity (Fig. 1). Thus complete rheological data for the lavas over a wide range of shear rates can be obtained (Fig. 2).

Two additional instruments were used to measure yield strengths on Etna[8]. One is a stainless steel shear vane attached to a torque wrench. After preheating, the vane is inserted into the lava and the torque is increased until the shear vane begins to rotate. Shearing resistance along the shaft is obtained by repeating the measurement at the same depth with the shear vane removed. Thus viscous resistance due to shearing within the lava surrounding the shear vane (and hence the yield strength of the lava) is readily calculated. The third instrument to be used on Etna was a penetrometer. This comprised a 2 m long, 3 cm diameter stainless steel rod, and a pressure transducer which was used to apply a known pressure during insertion. Two other methods were used to acquire rheological data on Etna. These involve (1) measuring the width of newly-formed levees[7,13]; and (2) measuring the dimensions of small lava flows[13,14]. Temperatures were measured during each rheological measurement using a Cr–Al thermocouple.

Rheological data and accompanying temperatures measured on Etna are given in Figs 2 and 3. Figure 2 shows the shear stress–shear rate data for a typical measurement using the Mark 2 viscometer. This measurement was made 3 m downflow from an active bocca at a depth of 0.2 m. The temperature of the lava

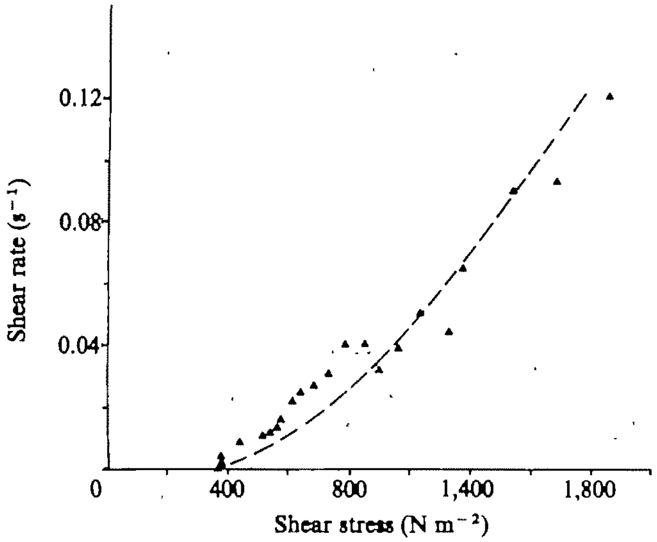

Fig. 2 Rheology of the 1975 Etna lava at 1,086 °C It can readily be seen that the Etna lava is pseudoplastic with a yield strength of 370(±30) N m^{-2}. If the curve is approximated to a Bingham relationship, the Bingham viscosity is 9,400 ± 1,500 Pa s.

at that point was 1,086 ± 3 °C. The results in Fig. 2 confirm that, at this temperature, the Etna lava had a yield strength of 370 ± 30 N m^{-2}, and it behaved in a pseudoplastic manner. If the curve is approximated to a Bingham relationship, the Bingham or plastic viscosity is 9,400 ± 1,500 Pa s at shear rates <0.15 s^{-1}. Lower plastic viscosities at higher shear rates are possible.

The yield strength of the interior of the 1975 Etna lavas was also calculated using the shear vane, the penetrometer and dimensions of initial levees[8]. Yield strengths measured by these methods are comparable with those calculated using the Mark 2 (Fig. 3), although the consistently higher values obtained by the conventional penetrometer suggest that shear resistance due to the cooler, outer margins of lava flows significantly affected the apparent yield strengths measured using this type of instrument. The relationship between the yield strength of the interior of the 1975 Etna lava and temperature are given by curve 1 in Fig. 3. Four estimates of yield strength were obtained from flow front dimensions. These measurements record the average or 'bulk' rheological properties of the lava which has a low yield strength in the hot interior and a high yield strength in the cool margins. The relationship between the bulk yield strength and the temperature of the isothermal interior of the Etna lavas is shown by curve 2 in Fig. 3. From curves 1 and 2 it seems that the bulk yield strengths of small advancing Etna lava flows are ~2.5 times greater than their hot interiors.

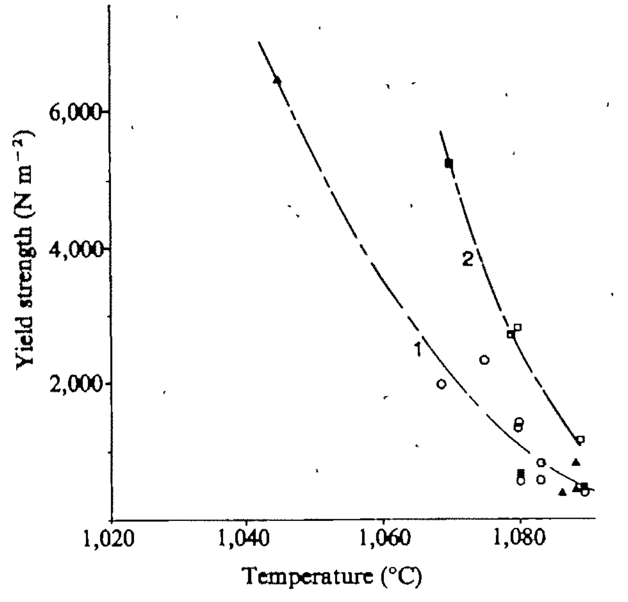

Fig. 3 Relationship between the central temperature and yield strength of specific points along several 1975 Etna lava flows. Curve 1 indicates the trend for the interior of lava flows, while curve 2 indicates the possible relationship for the fronts of advancing flows. It is postulated that curve 1 will tend, asymptotically, to a yield strength of zero at supraliquidus temperatures. ▲, Mark 2; ○, Shear vane; △, penetrometer; □, flow front data; ■, initial levee data.

Etna was the first volcano for which non-newtonian behaviour of lava was proposed[15]. Our results confirm that, on eruption, the 1975 sub-terminal Etna lavas were pseudoplastic with a yield strength. Compared with the tholeiite in Makaopuhi lava lake in 1968[4], the Etna lava flows had higher crystal contents (modal analyses: 35% for Hawaii and 45% for Etna) and lower effusion temperatures (1,135 °C for Hawaii and 1,090 °C for Etna). The higher crystal concentrations and lower effusion temperatures of the Etna lavas are, at least partly, responsible for the higher yield strengths and plastic viscosities of the Etna lava flows[3].

For the volcanologist, non-newtonian behaviour of lava has many implications, both in understanding the flow behaviour of lava flows and in interpreting their morphology. For example, as the apparent viscosity of a pseudoplastic material decreases with increasing shear rate, a fast-moving lava flow will seem to be more fluid than when it moves more slowly. Consequently, if the viscosity of lava flows is calculated from the commonly used Jeffrey's equation[12], Fig. 2 shows that lower apparent viscosities will be calculated for faster-moving lava flows.

For the petrologist, magma rheology is of considerable importance in many processes, such as ascent of magmas, crystal settling[16,17], convection in magmas and xenolith transport[18]. Depending on the reasons for the development of a yield strength in magmas, some, or all, of these processes may be affected by the possible non-newtonian properties of magmas. If the yield strength results entirely from the interaction effects of the phenocryst phase, then only relatively large-scale processes will be affected by the yield strength, for example the settling of xenoliths. On the other hand, if the yield strength results from the formation of a microcrystalline mosaic[19], liquid–liquid immiscibility[20], or structural reorganisation of the melt[21], small-scale processes will also be affected. In particular, crystal settling will not be possible in certain magmas. The reasons for the development of a yield strength in lava flows are investigated more fully elsewhere[22].

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Effect of degassing on rheology of basaltic lava

BEFORE eruption, most basaltic lava flows lose volatiles, either by Hawaiian—or Strombolian-type fire-fountaining. This gas loss may be of fundamental importance in explaining the observed non-newtonian characteristics of many lava flows. The model proposed here also helps to explain the variations in the surface morphology of many basaltic lava flows.

The loss of most common volatile components increases the viscosity of silicate melts[1,2], and this has been cited as a cause of moderate changes in the viscosity of lava flows[3]. However, an even more important consequence of gas loss is a sudden undercooling of the lava triggering an all-pervasive growth of quench crystals in the lava. This process leads to a rapid increase in viscosity and the development of a high yield strength in the lava.

Degassing of magma leads to undercooling of lava by three processes. First, decompression of the gas phase cools the total magma system adiabatically. For water, $\sim$20 °C cooling occurs per 1 wt% released (ref. 4 and L. Wilson, unpublished calculations). Second, the solution of gases suppresses the liquidus of a melt and alters the order of appearance of mineral phases[5]. Hence, sudden gas loss upsets equilibrium and the melt will generally become undercooled with respect to its anhydrous liquidus. Experimental studies on water solubility and the effect of increasing water pressure on liquidus temperatures[2,5,6] indicate that common types of basalt which contain 1% water would be saturated at 300–400 bar. Such a basalt would be undercooled, by this process, between 30 and 50 °C, if brought rapidly to the surface and then degassed. Carmichael *et al.*[7], on the basis of thermodynamic arguments, also deduced that the crystallisation temperatures of silicate phases would increase on volatile loss. Third, fire-fountaining results in some mixing with the atmosphere and opportunity for radiative heat loss. Some or all of this cooled magma may then be erupted as a lava flow. Swanson and Fabbi[8] have shown that fountain height increases with greater volatile content in Hawaiian fountains which fed lava flows. Consequently, the high fountains associated with large volatile contents would result in increased atmospheric cooling. Data from chilled pillow margins indicate that mid-ocean ridge basalts have water contents ranging from 0.25 to 1.0% with a tendency for alkali basalts to have higher contents than tholeiitic basalts[9–11], whereas basalts from calc-alkaline island arcs and back-arc basins seem to contain even higher water contents[12,13]. Taking a mean water loss from basalts of 1 wt%, we estimate that, as a result of the above three mechanisms, this loss would, typically, cause the lava flow to become undercooled by at least 50 °C. The amount of undercooling will increase with increasing gas loss.

Kirkpatrick[14–16] has shown that crystal nucleation and growth rates both increase with higher values of undercooling of a melt, attain a maximum at several tens of °C, and then decrease. For example, maximum nucleation and growth rates in basalt occur at 50–100 °C undercooling, that is, the same range of undercooling which we predict results from gas loss in many basalts. From Kirkpatrick's studies we estimate that plagioclase crystals will grow at rates of 0.1–1.0 μs^{-1} in a lava undercooled by 50 °C. Using a conservative value of 0.1 μs^{-1}, a 20 μm crystal could grow in 3.5 min. Growth and nucleation will continue until the residual melt has attained equilibrium. With large undercoolings the rapid growth of an extensive mosaic of crystallites can be anticipated in the early stages of flow. It also follows that strongly undercooled lavas (high original gas content) will require more extensive crystallisation to re-establish equilibrium at the extrusion temperature. As basalt lava flows commonly have emplacement times of hours, days or even weeks, gas loss thus results in an all-pervasive growth of quench crystals in the early stages of flow.

This mechanism is, therefore, far more effective in changing lava rheology than atmospheric cooling which penetrates into lavas only slowly because of their poor thermal conductivity[18–23]. In addition, crystallisation changes the composition of the residual melt which, in most cases, would become more siliceous. This effect, together with the sudden appearance of crystal dispersants and the accompanying loss of volatiles, would result in a greatly increased viscosity. More important, the lava would develop a yield strength. An aphyric magma which, before eruption, may have behaved in a newtonian manner will change to non-newtonian behaviour (develop a yield strength) as quench crystallisation takes place. A lava containing many phenocrysts will increase both in yield strength and viscosity.

Several morphological features of lava flows have been interpreted as resulting from non-newtonian rheology[18,24,25]. The formation of levees and channels and the ability of lavas to cease to flow on steep slopes has been attributed to the yield strength of lavas[18,25]. The formation of *aa* surfaces has been interpreted as being due to the inability of surface tension forces to overcome the yield strength[18].

Although lava flows with *aa* surfaces and levee and channel morphology are characteristic of high yield strength lavas, pahoehoe surfaces have also been observed forming on similar lavas, for example, Etna, 1975 (refs. 20, 23). On Etna the main factor controlling the type of lava which formed was effusion rate, pahoehoe surfaces forming on the low effusion rate lava flows. However, this generalisation cannot be extended to all other volcanoes as pahoehoe surfaces can also form on extensive flows with high inferred effusion rates[26]. Apparently therefore, when lava flows have a moderate to high yield strength, pahoehoe lavas form only at low effusion rates, whereas *aa* lavas form when effusion rates exceed a critical value (2×10^{-3} $m^3 s^{-1}$ on Etna in 1975). On the other hand, when lavas behave as newtonian fluids, or when their yield strength is low, pahoehoe flows form, even at high effusion rates. Although the yield strength of the Etna lavas results, at least partly, from their high phenocryst content, certain flows with a low phenocryst content show features indicative of a high yield strength. Examples are the 1961 Askja basalt lava[27] and the 1875 Sveinagja basalt[28].

These paradoxes in the morphology of basalts can be resolved by the mechanism outlined here. Magmas with high volatile contents will produce strongly undercooled degassed lava. These lavas will develop a high yield strength due to quench crystallisation and will form channelled *aa* flows except at low effusion rate. Magmas with low volatile contents will not be strongly undercooled so will not change rheology substantially on extrusion; they would have either low yield strengths or even behave as newtonian fluids. Formation of pahoehoe flows, even with high discharge rates, would result. Evidence for high volatile content is indicated in the Askja and Sveinagja eruptions directly, by the intense fire-fountaining observed and, indirectly, by the evolved nature of the basalts indicating extensive fractionation of anhydrous phases[28]. It is interesting that the 1961 Askja lavas turned from *aa* to pahoehoe flows later in the eruption, when the intensity of fire-fountaining waned.

The most sluggish basalts are commonly found in island arc settings. These basalts are generally much more viscous than basalts associated with the spreading environments. They invariably produce *aa* or block lava flows and are usually accompanied by violent fire-fountaining, for example, the 1971–72 eruption of Villarica, Chile. Calc-alkaline basalts are

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generally believed to have high water contents[12,13,29] compared with other common basalt types. Our proposal qualitatively explains these relationships. These basalts are more strongly undercooled after gas loss and consequently have much higher yield strengths and apparent viscosities.

There are several implications of these proposals. First, models of lava flow movement will be required to consider quench crystallisation due to gas loss as a major cause of downflow changes in rheology. Second, the rheology of active lava flows will generally not reflect the rheology of the magma before eruption, and there is a fundamental change in rheology accompanying gas loss. Hence, although many lava flows are non-newtonian this does not necessarily imply that all ascending magmas are non-newtonian. Finally, deductions based on the yield strength of lava from studies of flow morphology cannot be related simply to magma composition[18,30], but must also be related to gas content.

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Pre-ceramic maize on the north-central coast of Peru

An archaeological site, located near the beach line in a desert part of the Huarmey Valley, 292 km north of Lima, in the department of Ancash, province of Casma, district of Huarmey, 78°10′21″W, 10°02′45″S in Peru, was first observed by D. H. Kelley in 1957. It had been previously found by E. P. Lanning who identified it as a pre-ceramic site. It was first designated Huarmey North 1 and it appears as such in previous literature; we are assigning it its real name, Los Gavilanes. We report here ^{14}C dating of maize plant, tassel and cob material from this pre-ceramic site in Huarmey, Peru, at 3,750 ± 100 yr BP.

Charred stones of an underground structure where the associated maize material was found has a date of 4,800 ± 500 yr BP, by thermoluminescence analysis. The corn material was identified as being almost entirely of the Proto-Confite Morocho race, a modern counterpart of which exists today only in the central highlands of Peru. No evidence was found of maize races originating outside of the central Andean region.

A first sounding made by Kelley produced some maize material, which was examined by P. C. Mangelsdorf. Further excavations produced 238 different maize cob, tassel and plant parts. These findings were in a pre-ceramic context[1].

^{14}C measurements made on the maize material gave erratic results, which made the validity of the dating doubtful, so that a preliminary report[2] gave only general information, and no dating. It was found later that corn itself is an inadequate organic material for carbon dating, because growing corn has a most unusual rate of adsorption of ^{14}C and ". . dates of maize remains are known to be consistently too young . . . . This is due to the fact that *Zea mays* together with many other plants (mainly grasses) from semi-arid and arid regions utilise the recently discovered 4-carbon or Hatch–Slack photosynthetic pathway and not the more widespread 3-carbon or Calvin pathway for carbon dioxide fixation. Such C-4 species are not common in temperate regions; the syndrome generally constitutes an adaptation to a hot and dry environment" (ref. 3). It was also found that the technique of handling and treating the maize material presumably had not been appropriate to eliminate contamination by masses of crystallised carbonates which were later found adhering to the surface of many corn cobs at the site, and which could have obscured the radio-carbon results.

In 1974, with support of the Royal Ontario Museum of Canada, a new excavation was made, which yielded the results presented here and which permitted us to re-evaluate earlier information[2] and prepare a new report[4]. The site is located on the right of the Huarmey river banks, bordering on a fossil lagoon, which was formed between barriers of old beach lines over an old stream bed. Alyssian winds cause constant movement of shifting sands that have covered the site completely. On the surface only stone accumulations are visible, some of which could be circular structures, arranged in a confusing pattern.

The 1974 excavation was made in one of the circular structures. This was only partially completed because of the large volumes of sand that had to be removed. The excavation yielded an irregular inverted truncated cone, the walls of which are built with irregular stones, 9 m in diameter at the top, and 1.30 m deep. On the walls of this structure abundant residues of maize leaves were found. A cut on one of the sides of the structures made in order to establish its nature, showed abundant refuse mixed with the stone.

Preliminary analysis has also shown the existence of human and animal bones, predominating among the latter are a shore and sea fauna, with scarce representation of *Camelidae*, *Cervidae* and *Canidae*. There is also abundance of molluscs, primarily *Bivalvia*, *Gasteropoda* and *Amphineura*. Plant remains of *Distichlis spicata* L., *Schirpus* sp., *Tillandsia* sp., *Inga* sp., *Pachyrrizus* sp., *Schinus molle* L., *Gossypium barbadense* L., *Cucurbita* sp., *Lagenaria siceraria* (Mol.) Standl, and *Ambrosia* sp. were also found.

The artefacts found at the site are mostly lithic. Tentatively, artefacts can be distinguished as made from cobblestone: these are basically strikers, choppers and chopping tools. Circular flat pebbles, worked along the edges by unifacial percussion and whose function is still unknown, are also characteristic and distinctive. Tools obtained from flakes are scrapers, notched pieces and some cores were used like scrapers; there is also a sizeable proportion of flakes used without further preparation. There are also textile fragments made by the technique of twining, typical of the pre-ceramic period. Some coprolites were also found and these are being studied at present.

On the basis of the charcoal pieces found associated with the debris, a ^{14}C dating was obtained of 3,750 ± 110 yr BP (GIF 3564, Laboratoire du Radiocarbone du Commisariat à l'Ener-

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gie Atomique CNRS). Charred stones of the excavated structure were dated by thermoluminescence analysis at 4,800 ± 500 yr BP (BOR 20, Laboratoire de Cristallographie et Physique Cristalline, University of Bordeux).

All evidence points to the Los Gavilanes site as belonging to the final Peruvian pre-ceramic period, that described by Lanning[5] as Pre-ceramic 6, and in which corn apparently played an important part.

Abundant maize cob, stalk and tassel material was recovered from the 1974 excavation made at the Los Gavilanes site. Nine intact corn cobs and 12 cob fragments were examined and measured (Fig. 1). They ranged in length from 2.5 to 9.4 cm, with diameters ranging from 0.7 to 1.4 cm. About 70% of the ears and cob fragments have 8 rows of kernels, with all the remainder having ten rows except one with 12 rows. The cupules of the cobs are distributed rather laxly, are rather deep, and are 2.5 mm in width and 4.5 to 6.0 mm in length, some with prominent cupule wings, pilosity of the cupules mostly low to medium, but a few with high pilosity. The kernels may have been isodiametric, about 3 mm in size, of the popcorn type, with 12 to 13 kernels in each row. The glumes are rather long, the external ones being semi-hard, coriaceous and the internal ones very soft and papery; they are either a quarter or half tunicate. Their colour is brownish red, and a few are purple (probable allele arrangement A_1A_2BP1).

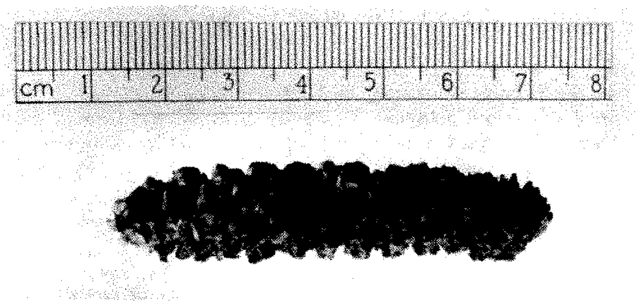

Fig. 1 Corn cob from Los Gavilanes site.

Husk material has a venation index of 7 to 9, the veins being very prominent. This is a rather high index[6], representative of primitive maize material. One of the husks exhibits a very definite purple colour. This colour type is prevalent today in the highland area of Peru, and has been shown[7] to have a definite selective advantage at high altitudes. Its presence in high frequencies in the Los Gavilanes population is a good indication of possible transfer of ancestral seeds from the highlands to the coast of Peru.

The cob peduncle is rather slender as in most highland popcorns. Stalk diameter is small, between 0.6–0.8 cm, and about 0.85 cm including leaf sheath.

Tassel fragments (Fig. 2) indicate a very lax type, such as found today in both primitive and advanced highland maize races of Peru. Condensation index of the tassels[6] ranges from 0.59 to 1.2, with the majority of the tassels falling within the shorter range of 0.58–0.8.

Unquestionably there is a single predominating maize race in this complex, an early form of Confite Morocho, designated as Proto-Confite Morocho[6]. A few cobs exhibit a fair degree of introgression of another race Proto-Confite Chavinense, better shown in one ear with some fasciation, and the only one in the cache to have 12 rows.

The abundance of plant and tassel material at the site would seem to indicate that the complete plants were harvested in the cultivated part of the valley and brought to the site tied in bundles, possibly carried on people's backs.

It is possible that the structure that has been opened was used as a silo. An interesting observation in this connection is that all the stones of the sides of the excavated structure have been burned. Similar storage pits lined with stones were found by A.G. full of ears of corn in sandy hills near Lima. The corn ears were protected from insects and rodents by filling the spaces between them in the pits with sand. Similar modern storage systems using sand were still in use in 1950 in the Huarmey Valley, and were examined at that time by A.G.

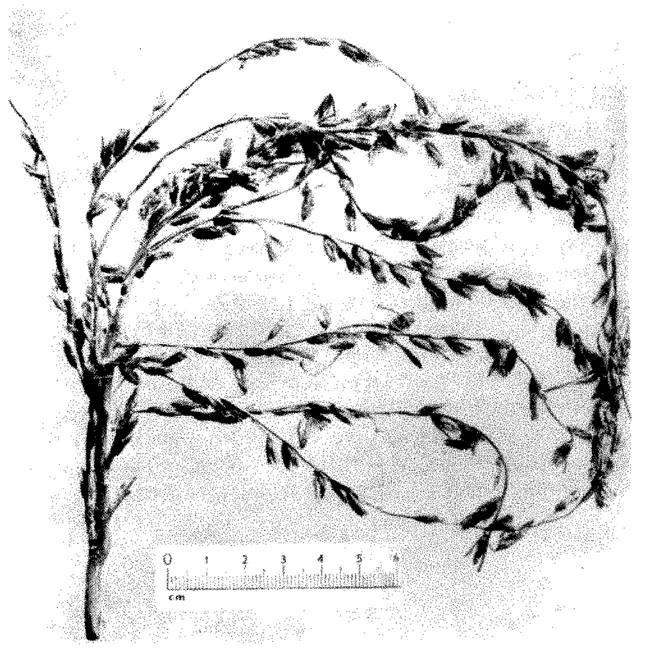

Fig. 2 Complete tassel from Los Gavilanes site.

The implication of the find is that maize was cultivated on the coast of Peru in the pre-ceramic period, about 4,000 years ago. This maize is undoubtedly related to the Confite Morocho race presently existing in the highlands of Peru and one of the most primitive races in existence. This find tends to confirm the postulation of both the origin of primitive coastal maize of Peru, as migrating from the Peruvian highlands[6], and the origin of cultivation and domestication of maize in the Central Andean region as an independent process from the domestication of maize in Meso-America[8]. There is no evidence on this site at this early period of introduction of maize resembling the primitive types that were being cultivated in Mexico contemporaneously.

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Lead detoxification by a copper-tolerant isopod

THE acquisition of metal tolerance by freshwater and marine invertebrates in environments polluted by mine waste in south-west England has now been established[1,2]. Generally, for invertebrates such tolerance has been shown to be specific for particular metals[1], although the literature suggests that co-tolerance may operate for copper and silver in the polychaete *Nereis diversicolor*[3] and for zinc and nickel in the grass *Agrostis tenuis*[4,5]. We show here that co-tolerance operates in the isopod crustacean *Asellus meridianus*, in which tolerance to copper seems to confer tolerance to lead. Two populations of *A. meridianus* from differing habitats are also shown to use different methods for achieving lead tolerance.

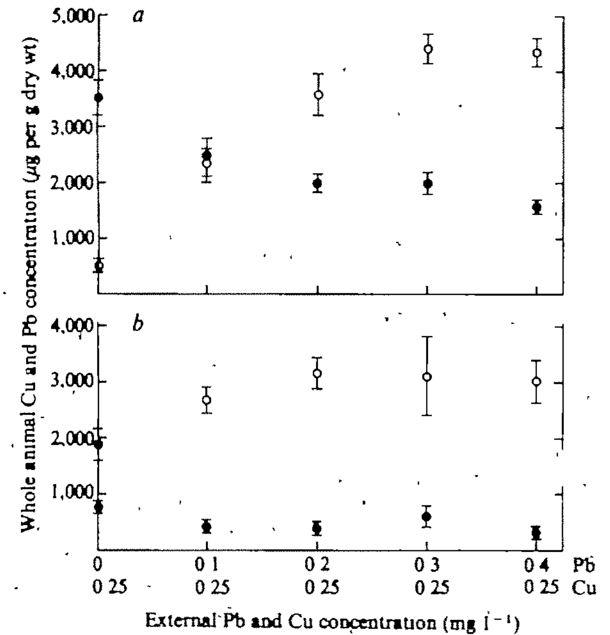

Fig. 1 The effect of increasing concentrations of lead (0.1–0.4 mg l^{-1}) and a constant copper concentration (0.25 mg l^{-1}) on uptake of copper (●) and lead (○) by *A. meridianus* from *a*, the Hayle and *b*, the Gannel, after 14 d exposure to test solutions at 15 °C±1 °C. Experimental techniques followed those described in detail elsewhere[2]. Test solutions were made up using Analar grade $CuSO_4$ and $Pb(NO_3)_2$ in a freshwater medium; solutions were renewed every 48 h throughout the experiment. Twenty animals were used at each dilution and batches of five whole animals were wet digested in Aristar HNO_3 and analysed for copper and lead with an Evans electroselenium 240 atomic absorption spectrophotometer. Each point is the mean of four batch samples; bars represent the s.e.m

In the present study, two populations of *A. meridianus* Rac were studied from two rivers, the Hayle and the Gannel, which, respectively, drain disused copper and lead mines in Cornwall, U.K. Previous work[2] had established copper and lead tolerance in animals from the River Hayle, where 'total' copper concentrations in the water range between 0.10 and 0.25 mg l^{-1} and levels of lead are low, <0.01 mg l^{-1}. A marked lead tolerance had also been observed in animals from the River Gannel, where total lead concentrations in the water range between 0.19 and 0.35 mg l^{-1}. No copper tolerance was observed in animals from the latter site, where total copper levels in the water are 0.01–0.04 mg l^{-1}.

Animals from both populations were exposed to solutions containing copper and lead alone and to mixed solutions containing the two metals in various proportions. The uptake of copper by Hayle animals is considerably reduced by the addition of increasing levels of lead, which are accumulated readily (Fig. 1*a*). Uptake of copper by the Gannel population in similar conditions is lower than that of Hayle animals and is apparently unaffected by the addition of increasing concentrations of lead. Lead uptake by these animals is also lower than that shown by the Hayle population (Fig. 1*b*).

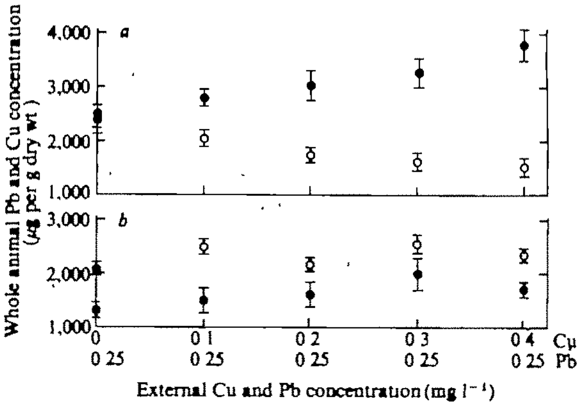

Fig. 2 The effect of increasing concentrations of copper (0.1–0.4 mg l^{-1}) and a constant lead concentration (0.25 mg l^{-1}) on uptake of lead (○) and copper (●) by *A. meridianus* from *a*, the Hayle and *b*, the Gannel, after 14 d exposure to test solutions at 15 °C±1 °C. Each point is the mean of four batch samples; bars represent the s.e.m.

When experimental conditions are reversed and animals are exposed to a constant lead concentration and increasing copper levels, uptake of lead by Hayle animals is reduced at higher copper concentrations (Fig. 2*a*). Reduction of the amount of metal accumulated by these animals is not as pronounced as in the previous experiment (Fig. 1*a*). Similarly, uptake of copper is not as marked as uptake of lead in the first experiment, suggesting that lead may be more preferentially bound than copper. Lead uptake by the Gannel population is apparently unaffected by increasing copper concentrations and copper uptake is low compared with that of Hayle animals (Fig. 2*b*).

Earlier work[2,5,6] had established that the hepatopancreas served as a principal storage organ for trace metals in isopod crustaceans. Ultrastructural and subsequent X-ray microanalytical studies of the hepatopancreas of *A. meridianus* from the Hayle exposed to lead suggest that both copper and lead are present at the same sites within the cells of the hepatopancreas (Fig. 3*a–c*). It is apparent that both copper and lead compete for sites in the hepatopancreas of this tolerant isopod, as both chemical analysis (Fig. 4) and microanalysis confirm a relationship between the two metals. X-ray microanalysis further demonstrates that lead is present only in the 'cuprosomes' of exposed animals. It is suggested that lead is more readily bound than copper, which is released into the lumen of the hepatopancreas, probably as a result of apocrine secretion (Fig. 3*a*) in which the apical end of the cell is lost together with the granules[8], in response to an incoming dose of lead. Similar preferential binding of lead over copper has also been observed in earthworm chloragosomes (Ireland, personal communication).

The ability of copper-tolerant isopods from the Hayle to 'detoxify' lead by storing the metal in 'cuprosomes' at the expense of copper indicates that metal tolerance in invertebrates may not be as specific as had originally been supposed. Also, the mechanisms operating for particular metals, in this case lead and

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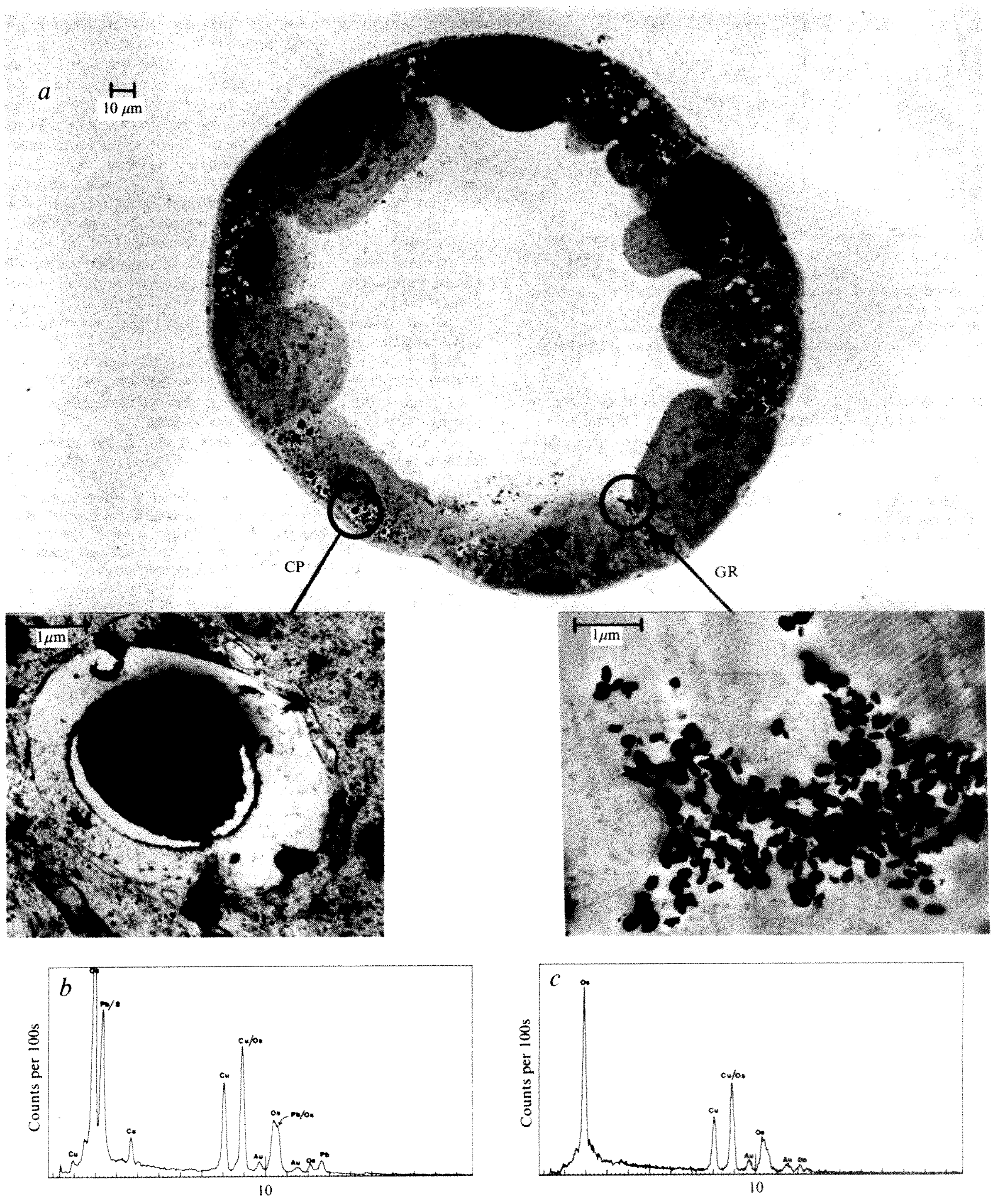

Fig. 3 Uptake of lead by isopods from the River Hayle exposed to 0.5 mg l^{-1} Pb for 10 d at 15 °C ± 1 °C. *a*, Photomicrograph showing 1-μm section of the hepatopancreas, stained with toluidine blue, of an exposed Hayle animal. Insets show electronmicrographs of densely staining 'cuprosomes' (CP) around the border of the cells and granular material (GR) being released into the lumen of the hepatopancreas. The hepatopancreatic caeca of lead-exposed animals were fixed in gluteraldehyde and osmium tetroxide, dehydrated and embedded in Araldite. 1-μm sections were cut and stained in toluidine blue and thin sections (600–1,000 Å) were then mounted on copper grids for ultrastructural analysis. The sections were viewed in an EM6-b (AEI Scientific Apparatus) electron microscope. *b*, The elemental spectrum of a cuprosome (CP) of a lead-exposed animal, showing peaks for Os (fixative), Au (grid) and constituents (Pb, Cu, Ca, S) of cuprosome. *c*, The elemental spectrum of granular material in the lumen of the hepatopancreas of a lead-exposed animal showing peaks for Os (fixative), Au (grid) and constituents of granular material (Cu). Hepatopancreatic caeca were fixed as above and sections (600–1,000 Å) mounted on gold grids for analysis by the electron microscope micro-analyser EMMA 4 (AEI Scientific Apparatus). Full vertical scale is 18,300 counts; in both cases the displayed spectrum takes into account the spectrum for the surrounding cytoplasm, which has been subtracted from the original.

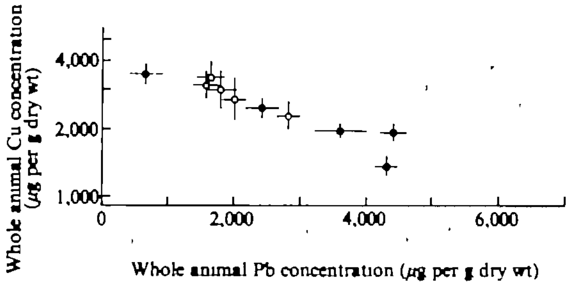

Fig. 4 Levels of copper plotted against levels of lead accumulated by whole animals from the Hayle during exposure to copper and lead alone and to mixed solutions of copper and lead. ●, Points obtained during exposure of animals to a constant Cu concentration and increasing Pb concentrations; ○, points obtained during exposure of animals to a constant Pb concentration and increasing Cu concentrations Each point is the mean of four batch samples; bars represent the s.e.m.

copper, in different populations of the same species are not necessarily similar, as indicated by the ability of Gannel animals to restrict the uptake of both copper and lead into the hepatopancreas.

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Transient tritanopia experiment in blue cone monochromacy

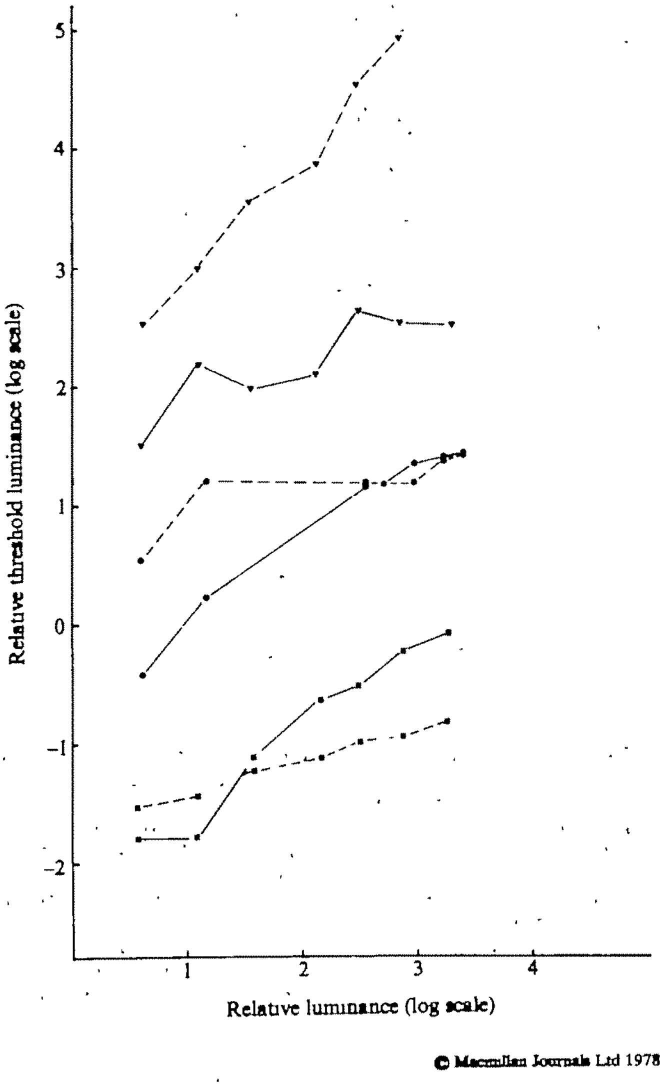
WHEN the eye has been adapted to a bright yellow light, a marked loss of sensitivity to short-wavelength stimuli may be recorded immediately after the extinction of the adapting field. This phenomenon, first described by Stiles[1], was termed transient tritanopia by Mollon and Polden[2]. They supposed that a recovering long-wavelength mechanism may inhibit or otherwise suppress the blue cone signal during early dark adaptation. Transient tritanopia was found in one protanope and, in a modified form, in one deuteranope. This indicates that each of the red or green mechanisms or both in combination may give rise to the inhibition effect. It is of particular interest in this connection to know if this kind of inhibition is present in persons without red or green cones. In blue cone monochromacy, a unique kind of colour vision anomaly, there are only blue cones and no functioning red or green cones. We report here the experimental results obtained with such a person where no suppression effect upon the blue mechanism could be demonstrated. The results are compared with those of a typical rod monochromat and a person with normal vision.

The blue cone monochromat (BTW), a 17-yr-old male, had visual acuity of 6/60 in each eye. He had pendular nystagmus and moderate photophobia. The presence of functioning blue cones was ascertained during the clinical examination and by spectral sensitivity studies[3]. By means of two modified Prado Universal projectors, a monochromatic circular test spot of 450 nm and 1° angular size was presented on a 30° yellow adapting field projected on a $BaSO_4$ screen. The viewing distance was 0.40 m. The test spot was presented about 1° above a fixation mark. The field of adaptation was produced by a Wratten 12 gelatine filter. Every 18 s the adapting field was extinguished for 3 s and a single test flash, lasting 30 ms, was presented 400 ms after the extinction of the field. Stimulus duration was controlled by electromechanical shutters. Maxwellian-view presentation was not preferred because of the subject's nystagmus. Preliminary experiments did not show significant differences either using artificial pupils or with maximally dilated pupils. The experiments were therefore performed with natural pupils. During the short period of 400 ms after extinction of the adapting field the pupil is not likely to show any significant change[4].

At photopic adaptation levels, the normal observer shows a distinct loss of threshold sensitivity in the dark interval. The blue cone monochromat subject, however, showed no significant loss of sensitivity at any level of adaptation (Fig. 1).

A slight increase of threshold sensitivity at lower adaptation levels is seen in the normal observer and is due to rod intrusion

Fig. 1 The transient tritanopia experiment in three kinds of observers: a normal trichromat (■), a blue cone monochromat (●) and a rod monochromat (▼). Ordinate, relative threshold luminance at which the blue test spot is detected; abscissa, relative luminance of adapting field. - - - -, log threshold luminance recorded on a steady yellow field. ——, log threshold luminance 400 ms after the adapting field has been turned off. Luminance is given relative to 1 cd m^{-2}. The curves for the blue cone monochromat and the rod monochromat have been displaced 2 and 4 log units respectively along the ordinate axis.



consequent to the parafoveal stimulation. This increase of sensitivity is more pronounced in the blue cone monochromat, probably because his nystagmus causes a greater participation of rod receptors.

As rod activity thus influences the response functions, it is of interest to study the results obtained in the same conditions in a rod monochromat, that is, a person without ordinary cones. The results of the rod monochromat are shown in Fig. 1 by the top curves. At low adaptation levels, the response pattern is similar to that of the blue cone monochromat, but it differs significantly at higher adaptation levels. The ratio between threshold values in the light and dark intervals increases with the luminance of the background light. At high luminances the threshold sensitivity regains more than 2 log units during the short period of 400 ms after extinction of the background light. This fast recovery rate is essentially different from the responses of the two other observers having both cones and rods in their retinas.

When acting without the influence of other receptors, the rod mechanism, thus has a very great ability of recovering after exposure to the yellow light. By the presence of blue cones this ability of fast recovering is completely lost, as the threshold values are now at the same level irrespective of the background light being on or off. When green and red cones are present, there is also a suppression of the blue mechanism giving further loss of threshold sensitivity in the dark interval. This, which is the transient tritanopia, is demonstrated by the normal observer.

The lack of transient tritanopia in the blue cone monochromat supports the theory of Mollon and Polden that this phenomenon in normal persons is an effect due to the inhibition of the blue cone mechanism by the other cones.

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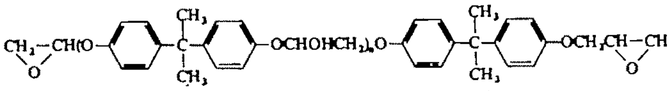
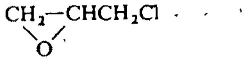
Mutagenic action of aromatic epoxy resins

EPOXY RESINS are used extensively for protective coatings and in paints and adhesives; the consumption of epoxy resins during 1973 was 190,000 metric tons, which is an almost twofold increase over a period of 10 years[1]. About 90% of the epoxy resins used are manufactured by condensation of two molecules of epichlorhydrin with one or more molecules of bisphenol A (2,2-bis (4-hydroxyphenyl)-propane). The mutagenic action of simple epoxides such as ethylenoxide and epichlorhydrin was first shown in fruit flies[2] and has subsequently been demonstrated in a wide range of organisms. Epoxy resins are diepoxides and bifunctional alkylating agents, and bifunctional alkylating epoxides are known to be mutagenic[3,4]. Mutagens are suspected of having a carcinogenic effect because of the relationship between carcinogenicity and mutagenicity[5] and most diepoxides are carcinogenic in mice and rats[6]. We report here that aromatic epoxy resins are mutagenic in *Salmonella typhimurium*, and may thus represent a cancer risk in man.

Table 1 Compounds tested by the *Salmonella*/microsomé mutagenicity test



			MW	*n*
Epikote	828	(E 828)	370	0
Epikote	1001	(E 1001)	950	1
Epikote	1004	(E 1004)	1,800	3
				
Epichlorhydrin		(ECH)		92.5

The Epikote resins were supplied by Shell Chemicals According to that firm, Epikote 828 is basically the diglycidylether of bisphenol A, as indicated by the formula, plus a small proportion of higher molecular weight material. The higher molecular weight resins contain some resin of lower molecular weight. Epichlorhydrin was from Fluka.

Three aromatic epoxy resins were tested by the plate incorporation assay with histidine-requiring mutants of *S. typhimurium*. The test was carried out as described by Ames *et al.*[7]. Epichlorhydrin (ECH) was included as positive control (see Table 1). The epoxy resins are all mutagenic in tester strain TA 100 (Fig. 1).

To ensure that mutagenic action was not due to interaction between the resins and dimethyl sulphoxide (DMSO), which is normally used as solvent in the plate incorporation assay, we also tested an aqueous emulsion made by ultrasonic emulsification of E 828. The aqueous emulsion was mutagenic to *S.typhimurium* TA 100, but to a lesser extent than in assays where DMSO was used as solvent.

E 828 was the most active compound of the resins tested, and both E 828 and E 1001 were more mutagenic than the positive control, ECH. The solubility of the resins decreases as the molecular weight increases. The resins were suspended in the agar medium in the amounts shown in Fig 1. However, as the resins were not in solution, the amounts given may not express the true doses.

Comparison of the first column of Table 2 with Fig. 1 shows that the mutagenic action of ECH in tester strain TA 1535 was about the same as that demonstrated with TA 100. In contrast, the mutagenic activity of the resins was very low compared with that observed using TA 100. This indicates that the mutagenic action of the resins is not caused solely by the possible content of unreacted ECH in the resins.

Assays were carried out to determine the mutagenic effect of epoxy resin metabolites. After addition to the plates of rat liver microsomes, prepared from rat liver activated with sodium phenobarbital and containing NADP, the mutagenic activity of the epoxy resins and ECH was reduced almost 10-fold in assays with TA 100.

When the same assays were carried out with TA 1535, E 828 was clearly activated by rat liver enzymes in the presence of NADP (compare the second and first columns in Table 2). In contrast, this resin was inactivated in assays where NADP was omitted (see third column in Table 2). The results obtained with E 1001 showed the same tendency.

As in the assays with TA 100, the mutagenic activity of ECH in assays with TA 1535 was reduced almost 10-fold by rat liver enzymes (see Table 2). This reduction in activity was independent of the presence of NADP. The inactivation of epoxy resins and ECH by microsomes without NADP was enzymatic, since the addition of heat-inactivated microsomes to the plates gave the same results as shown in the first column of Table 2.

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It is known from studies of polyaromatic hydrocarbons and styrene oxide that the microsomal epoxide hydrase catalyses the hydration of epoxides to diols, and that the soluble fraction glutathione-*S*-epoxide transferase catalyses the conjugation of epoxides with glutathione[8]. These two enzymes are active in rat liver preparations without NADP. The resulting metabolites are considered to be non-mutagenic or less mutagenic than the epoxide[9,10].

The activation of the aromatic epoxy resins as found in TA 1535 is not caused by decomposition of the compounds and liberation of bisphenol A. Bisphenol A was tested for mutagenicity, both with and without the addition of liver microsomes and was not active in tester strains TA 1535 or TA 100.

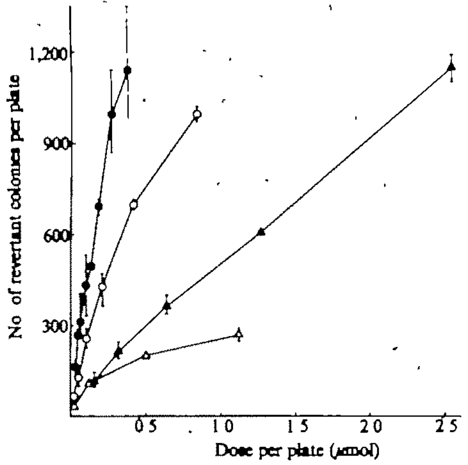

Fig. 1 Mutagenicity of aromatic epoxy resins and ECH for *S. typhimurium* TA 100 (revert by base-pair substitution). The epoxides were dissolved in 100 μl dimethyl sulphoxide (Merck, spectrometric grade). Bacteria and dissolved test agent were incorporated into the agar overlay. No S-9 mix was added. The number of spontaneous revertant colonies was estimated at 250. Each point is an average of numbers scored on three plates. The limits of the vertical lines indicate the lowest and highest values obtained with each dose. ●, E 828; ○, E 1001; △, E 1004; ▲, ECH (positive control)

Extensive studies of benzo(*a*) pyrene metabolites show that the combined epoxide hydrase–monooxygenase system leads to the formation of highly mutagenic diol-epoxide[11]. The monooxygenase system is dependent on NADP. Metabolites of this type might explain why NADP-dependent enzymes play a role in the formation of metabolites which are more mutagenic than the original epoxy resins to tester strain TA 1535, as shown in Table 2.

The demonstration of a mutagenic effect of aromatic epoxy resins in *S. typhimurium* indicates a genetic hazard, including a cancer risk, for humans exposed to these compounds. The fact that epoxy resins can be metabolised to mutagenic active compounds enhances the genetic risk from aromatic epoxy resins. Man is exposed to the resins during their manufacture and use either by skin contact with the compound or by inhalation of air contaminated with droplets or powder particles of epoxy resin. It has been demonstrated that topical aromatic epoxy resins (MW 350) are toxic to rats[12]. Therefore, it seems that the resins can penetrate the skin and be absorbed by animals.

Aromatic epoxy resins have been tested for carcinogenicity. After one skin exposure to 900 μM aromatic epoxy resin (MW 395), one skin tumour and three malignant lymphomas appeared in 50 mice[13]. In a study with only 40 mice, no tumours were found after skin painting with undiluted resin[14] but results are inadequate since none of the animals was alive after 24 months. In a study by Hine *et al.*[15], four malignant sarcoma tumours were produced at the site of injection after administration to 30 rats.

Table 2 Activity of aromatic epoxy resin metabolites ascertained by the *Salmonella* microsome mutagenicity test

Compound	Dose (μmol per plate)	−S-9 mix	+S-9 mix	+S-9 mix (−NADP)
E 828	0.54	ND	39	ND
	1.35	ND	109	ND
	5.40	58	521	ND
	13.5	79	996	20
	54.0	162	ND	14
E 1001	2.1	24	39	9
ECH	0.254	216	ND	ND
	0.635	474	ND	ND
	2.54	1,418	34	35
	6.35	ND	152	105
	25.4	ND	911	731

The figures represent the numbers of revertant colonies per plate produced by various doses of aromatic expoxy resins and epichlorhydrin (ECH) in tester strain TA 1535 (reverting by base-pair substitution). The spontaneous revertents (36) are subtracted. Each figure is an average of numbers scored on 3–10 plates in two independent assays. ND, not determined. The plate incorporation assay described by Ames *et al*[7]. was followed without modification. The assay was performed with and without the addition of rat liver microsome preparations. To some plates, preparations containing NADP were added, that is S-9 mix described by Ames *et al.*[7]. In other plates S-9 mix without NADP was used. 150 μl S-9 was added to each plate.

Since malignant tumours were demonstrated, and as the number of animals was small and the period of observation short in the above-mentioned studies, there is no basis for concluding, as did Hine *et al.* and others, that aromatic epoxy resins are safe from the point of view of carcinogenicity.

Owing to the long latent period for genetic damage and most cancers in humans[16], it is not possible to identify the genetic or cancerogenic hazards of aromatic epoxy resins by epidemiological studies in the immediate future, since consumption on any scale may not have taken place until the 1960s.

To prevent cancer and genetic damage in humans it is necessary to minimise the exposure to substances such as aromatic epoxy resins which have been shown to be mutagenic in bacteria, and thus must be considered as potential mutagens and carcinogens in human beings.

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T cell origin of human macromolecular insoluble cold globulin

THYMUS-DERIVED (T) lymphocytes are a heterogeneous cell population in terms of their function and the surface antigens they express. In the mouse, a subpopulation of sensitised T cells is cytotoxic for allogeneic or modified syngeneic target cells by virtue of recognition of histocompatability antigens, that is, H-2K and H-2D (refs 1 and 2). These cytotoxic T lymphocytes express a specific antigen of the Ly antigen series, Ly-2,3 (refs 3 and 4). Another subpopulation of T cells mediate responses in the mixed lymphocyte reaction and the graft-versus-host reaction[4,5]. These cells recognise immune response associated antigen (Ia) and possess another antigen of the Ly series, Ly-1 (refs 4 and 5). In addition, separate classes of T cells mediate helper and suppressor regulatory effects on antibody response and also have distinctive Ly membrane markers[4,6,7]. Although membrane proteins such as immunoglobulin and B-cell antigens are specific to bone marrow-derived (B) lymphocytes in humans, there are few well characterised proteins that serve to distinguish human T cells[8-12]. We have recently identified a glycoprotein synthesised in mouse thymocytes and splenocytes[13,14]. This protein has been termed macromolecular insoluble cold globulin (MICG) to describe its major physicochemical properties. It has a molecular weight of 225,000, is insoluble in the cold in non-ionic detergents and has the electrophoretic mobility of a β globulin. MICG has also been shown to be the exclusive product of T lymphocytes[15]. This report describes the characterisation of a similar protein in human T cells.

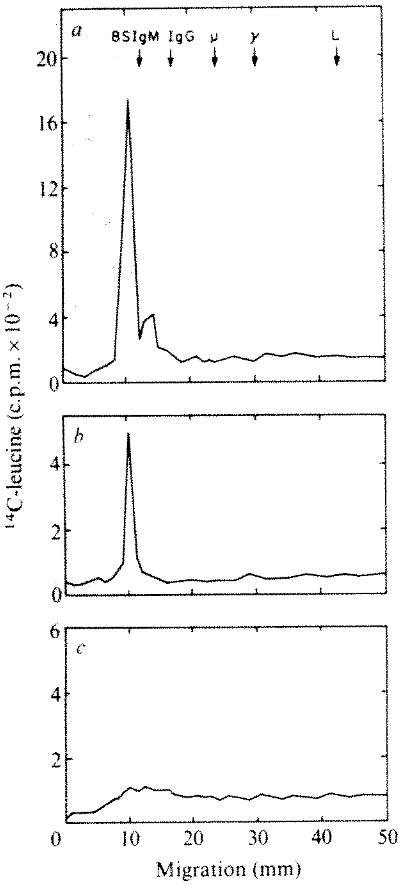


Fig. 1 Cold precipitation of ^{14}C-leucine labelled human MICG protein from lysates of: *a*, PBL (36×10^6); *b*, T cells (12×10^6); *c*, B cells (12×10^6). Precipitates were reduced and subjected to SDS-PAGE (5%). Top of gel is on the left side of figure. Arrows indicate the position of ^{14}C-leucine labelled markers (derived from mouse plasmacytoma cells of the appropriate class) which were electrophoresed on companion gels[13]. 8S IgM unreduced monomer IgM (MW 200,000); IgG, unreduced intact IgG; μ, IgM heavy chains; γ, IgG heavy chains; L, light chains.

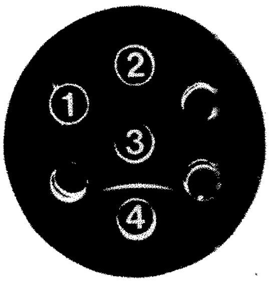
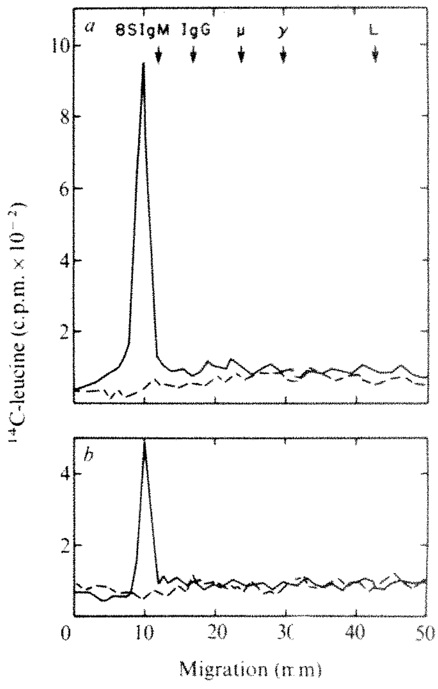


Fig. 2 Ouchterlony analysis (double immunodiffusion). Wells: 1, PBL lysate in 0.5% Triton X-100 and 2 M urea; 2, rabbit antiserum to human MICG; 3, rabbit antiserum to human cold insoluble globulin (given by M. Mosesson); 4, normal human plasma.

Peripheral blood lymphocytes (PBL) were separated from heparinised whole blood by Ficoll-Hypaque centrifugation (FHC)[16]. T lymphocytes were isolated from PBL by rosette formation with sheep red blood cells (SRBC) and sedimentation on FHC[17]. The lymphocytes from the SRBC pellet (after lysis of SRBC) were used as a source of T cells. They contained less than 2% surface immunoglobulin-positive cells and 97% of these cells formed rosettes with SRBC. B lymphocytes were isolated from the B cell-enriched fraction (unrosetted cells) at the Ficoll–medium interphase by application to Sephadex G-200 anti-human Fab columns[18,19]. Bound cells, eluted by competitive inhibition with human IgG, were 98% surface immunoglobulin-positive and less than 1% formed rosettes with SRBC. Surface immunoglobulin-positive cells were determined by fluorescence microscopy using rabbit anti-human light chain antiserum labelled with fluorescein isothiocyanate[20].

Fig. 3 Antiserum precipitation of radiolabelled lysates from PBL, T cells and B cells. Lymphocytes, labelled with ^{14}C-leucine, were lysed in isotonic buffer[13], *p*H 7.2 and 0.5% (v/v) with respect to desoxycholate, for 20 min at 4 °C. Lysates were immunoprecipitated using antiserum (250 μl) in antibody excess for 16 h at 4 °C. Samples were reduced and treated on SDS-PAGE (5%). *a*, PBL (75×10^6) precipitated with either anti-MICG antiserum (——) or normal rabbit serum (- - - -); *b*, T cells (40×10^6) (——) or B cells (40×10^6) (- - - -) precipitated with anti-MICG antiserum.



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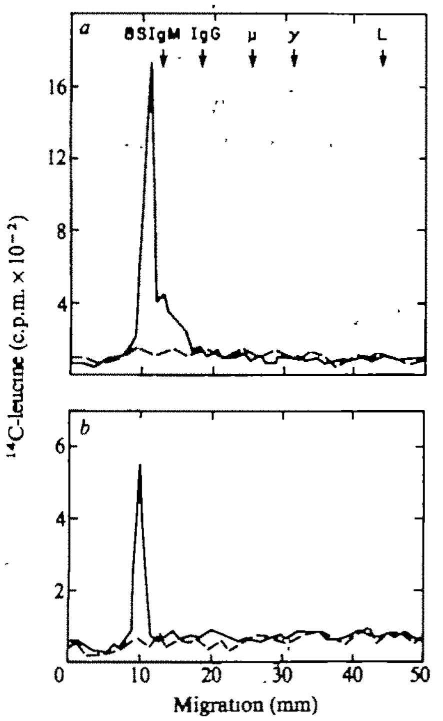

Fig. 4 Specificity of antihuman MICG antiserum. Radiolabelled (^{14}C-leucine) PBL lysates were precipitated and electrophoresed on 5% SDS gels after reduction: *a*, PBL (50×10^6) were lysed in NP-40 and cold precipitated (——) at 4 °C for 2 d and the supernatant reacted with anti-MICG antiserum (250 μl) in antibody excess (– – – –). *b*, Lysates from PBL (30×10^6), prepared as in Fig. 3, were reacted with either unadsorbed anti-MICG antiserum (——) or anti-MICG adsorbed with T cells (300×10^6 T cells per ml antiserum) for 1 h at 37 °C (– – – –).

Lymphocytes, radiolabelled with 10 μCi of ^{14}C-leucine, were lysed in Nonidet P-40 (NP-40) and the lysates cold precipitated for 2 d at 4 °C in detergent buffer[14]. The washed precipitates were dissolved in 2% SDS–8 M urea–phosphate buffer for 1 h at 37 °C, reduced with 0.5 M 2-mercaptoethanol and alkylated with 0.65 M iodoacetamide. The samples were then electrophoresed on 5% SDS–polyacrylamide gels, and the gels sliced and counted[14]. Cold precipitated PBL lysates demonstrated a major peak of radioactivity with a molecular weight of 225,000 (Fig. 1*a*), compared with appropriate standards[14]. Incubation of cell lysates for 2 d at 22 °C did not induce the precipitation of this macromolecule. Similar to mouse MICG, the 225,000 MW protein had the electrophoretic mobility of a *β* globulin.

To investigate the cell of origin of this protein, PBL were separated into T and B lymphocyte subpopulations and radiolabelled lysates analysed for cold insolubility. As shown in Fig. 1*b* and *c*, only isolated T cells were able to synthesise the cold precipitable macromolecule. There was no radioactivity cold precipitated from B lymphocytes.

The relationship between MICG and human serum proteins was analysed with antiserum to isolated MICG[14]. This antiserum did not react with isolated, radioiodinated proteins, including albumin, α_2 macroglobulin, transferrin, IgG, IgA or IgM, nor did it react with concentrated human plasma or serum in Ouchterlony analysis[14]. In contrast, antiserum to MICG showed a single precipitan band with PBL lysates (Fig. 2). Note also that a variety of monospecific antisera to human serum proteins did not react with PBL lysates in Ouchterlony analysis. These included antisera to α_2 macroglobulin, albumin, transferrin, IgM, IgA, IgG, IgD, IgE, fibrinogen, cold insoluble globulin and whole plasma (Fig. 2).

Monospecificity of this antiserum was also shown by direct precipitation of radiolabelled lysates derived from PBL, T cells and B cells. As MICG is insoluble in the cold in non-ionic detergents, it was necessary to lyse lymphocytes with a detergent in which MICG was soluble. When radiolabelled lymphocytes were lysed in Desoxycholate, the macromolecule remained in solution. After precipitation of these lysates with antiserum to MICG, a single peak of radioactivity was demonstrated on SDS gels for both PBL and T cell lysates (Fig. 3*a* and *b*). The molecular weight of this protein was 225,000, when compared with appropriate standards. In contrast, no radioactivity was precipitated from B cells with anti-MICG antiserum (Fig. 3*b*), nor did normal rabbit serum react with the radiolabelled lysates (Fig. 3*a*).

To determine whether the 225,000 cryoprotein visualised using SDS–polyacrylamide gel electrophoresis (PAGE) was similar to the protein precipitated by anti-MICG antiserum, radiolabelled PBL lysates were cold precipitated and the supernatant allowed to react with antiserum to MICG. Following cold precipitation there was no radioactivity precipitated from the supernatant with anti-MICG antiserum (Fig. 4*a*). The reverse of this experiment, whereby radiolabelled lysates were first precipitated with anti-MICG antiserum and the supernatant cold precipitated, also demonstrated antigenic similarity (not shown).

As a final proof of the specificity of this antiserum for T cells, the antiserum was adsorbed with T cells and reacted with radiolabelled PBL lysates. In these conditions, the antiserum was not reactive with the radiolabelled lysate (Fig. 4*b*). When the antiserum was adsorbed with B lymphocytes, there was no loss of activity against PBL.

The present experiments demonstrate a unique protein present in human T cells. It has similar chemical characteristics to mouse MICG and has been termed human T-MICG. Further studies are in progress to elucidate the biological function of this protein. Certainly, T-MICG serves to distinguish human T cells from B cells.

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Mode of coupling between hormone receptors and adenylate cyclase elucidated by modulation of membrane fluidity

THE hormone- and neurotransmitter-dependent adenylate cyclases represent transmembrane regulatory systems in which the receptor unit faces the outside of the cell and the cyclase catalytic unit faces the inside. When the hormone or neurotransmitter binds to the receptor, the catalytic unit of the cyclase is activated and produces cyclic AMP at the inner surface of the membrane[1]. It has recently become clear that some hormone receptors are permanently coupled to adenylate cyclase whereas others are not. We demonstrate here that the effects of modulating membrane fluidity on the rate of adenlyate cyclase activation can be used to distinguish between the two modes of coupling.

Activation of adenylate cyclase requires the simultaneous binding of hormone to receptor and intracellular GTP to the guanyl nucleotide regulatory subunit[2]. Furthermore, in the case of the β-adrenergic receptor the hormone signal is terminated concomitantly with the hydrolysis of GTP to GDP and inorganic phosphate at the regulatory site[3]. Thus for the β-adrenergic receptor, and perhaps generally, the continued presence of both hormone and GTP is essential to maintain the steady-state level of active adenylate cyclase[2,3]. This mechanism may also account for the observation that non-hydrolysable GTP analogues induce a permanently active state of the cyclase in the presence of hormone. When the latter occupies the GTP regulatory site, the GTPase step is blocked and the enzyme remains in its inactivated state[2,4,5].

Recent evidence suggests two alternative modes of interaction between the receptor regulatory unit and the catalytic cyclase unit. Either the receptor and the enzyme are mobile and float in the membrane[6-10] (in this case the activation of the catalytic moiety takes place during the encounter between receptor and enzyme) or the receptor and the enzyme are permanently coupled to each other, as a typical soluble regulatory enzyme composed of regulatory and catalytic subunits such as aspartate transcarbanylase (in this case the subunit interactions are similar to those of classic regulatory proteins in solutions). The first mechanism is described in the following scheme:

$$\begin{array}{l} \mathrm{HR+E} \xrightarrow{k_1} \mathrm{(HRE)} \xrightarrow{k_2} \mathrm{HR+E'} \\ \quad \uparrow\!\downarrow K_\mathrm{H} \\ \mathrm{H+R} \end{array} \qquad (1)$$

where H is the hormone, E the enzyme, R the receptor, k_1 the bimolecular rate constant, E′ the activated enzyme, K_H the receptor–hormone dissociation constant and k_2 the first order activation constant. (HRE) indicates the transient ternary complex, the hormone–receptor–enzyme. If the ternary complex is short-lived ($k_2 \gg k_1$), the encounter between hormone-bound receptor and catalytic unit becomes rate limiting. Such a coupling mechanism has been termed 'collision coupling'. The second mechanism (permanent coupling) can be described by the following scheme:

$$\begin{array}{l} \mathrm{HRE} \xrightarrow{k} \mathrm{HRE'} \\ \quad \uparrow\!\downarrow K_\mathrm{H} \\ \mathrm{H+RE} \end{array} \qquad (2)$$

where RE is the receptor–enzyme complex, H the hormone, k, the first-order constant of activation and K_H the dissociation constant of hormone to receptor–enzyme complex.

In these two schemes we have eliminated the deactivation process of the active enzyme species. This is achieved in hormone-dependent adenylate cyclases when nonhydrolisable GTP analogues such as guanylylimidodiphosphate (GppNHp) replace GTP in the adenylate cyclase reaction[2,5]. Therefore, in the presence of hormone and GppNHp, the enzyme becomes permanently active and accumulates according to[9,10]:

$$B = A\{1-\exp(-k_\mathrm{obs}t)\} \qquad (3)$$

In the 'collision coupling' model (equation (1)), A = total concentration of catalytic units and B concentration of the units activated at time t. k_obs Is the pseudo first-order rate constant given by:

$$k_\mathrm{obs} = k_1[\mathrm{R_T}]\frac{[\mathrm{H}]}{K_\mathrm{H}+[\mathrm{H}]} \qquad (4)$$

where $[\mathrm{R_T}]$ is the total receptor concentration, k_1 the bimolecular rate constant and the term $[\mathrm{H}]/(K_\mathrm{H}+[\mathrm{H}])$ expresses the fraction of hormone-occupied receptors.

The maximal number of the activated catalytic units in that case is independent of receptor concentration, as every receptor can activate an unlimited number of catalytic units through the collision process. It is apparent from equation (4) that k_obs is directly proportional to the receptor concentration.

For equation (2), A in equation (3) is the total concentration of receptor–enzyme and B the concentration of the activated receptor–enzyme unit at time t. K_obs In this case is given only by:

$$k_\mathrm{obs} = \frac{k[\mathrm{H}]}{K_\mathrm{H}+[\mathrm{H}]} \qquad (5)$$

where k is the first-order rate constant of activation and the term $[\mathrm{H}]/(K_\mathrm{H}+[\mathrm{H}])$ is the fractional occupancy of the receptor by the hormone. At saturating hormone concentrations this term is equal to one.

We have recently shown[9,10] that the coupling of turkey erythrocyte adenylate cyclase to the β-adrenoceptor is 'collision coupling' whereas the adenosine receptor is permanently coupled to the enzyme in the same cell[11,12]. This was based on the observation that progessive destruction of the β-adrenergic receptor by a specific affinity label results in a proportional decrease in the rate constant of cyclase activation by the receptor, without reduction in the total number of catalytic units which can be activated. In contrast, progressive destruction of the adenosine receptor by a specific affinity label results in a proportional decrease in the number of catalytic units which can be activated without a change in the rate constant of activation[12]. As the collision coupling mechanism, in contrast to the precoupled mechanism, necessitates a relative movement of the receptor with respect to the catalytic units in the membrane matrix, a different dependence of enzyme activation by the hormone through membrane fluidity is expected for epinephrine compared with adenosine.

To test this hypothesis we modified the fluidity of turkey erythrocyte membranes and measured the kinetics of enzyme activation by epinephrine and by adenosine. Turkey erythrocyte membranes were fluidised progressively by the insertion of *cis*-vaccenic acid which is one of the most efficient fluidising agents[13]. The apparent membrane microviscosity was measured using fluorescence polarisation[14], using 1,6-diphenyl(1,3,5)hexatriene (DPH) as the fluorescence probe. In parallel, we measured the time course of adenylate cyclase activation to its permanently active state, by either *l*-adrenaline or adenosine, in the presence of saturating concentrations of GppNHp. The first-order rate constants for the first-order process of adenylate cyclase activation by the two hormones were derived from equation (3) according to the procedure described elsewhere[9,10].

Figure 1 shows that the rate constant of the epinephrine-dependent activation increases linearly with membrane fluidity, whereas the rate constant of the adenosine-dependent activation is independent of membrane fluidity. This supports the kinetic experiments described above. Furthermore, the linear depencence of k_obs on $1/\eta$ in the case of the adrenaline-dependent activation, strongly suggests that the interaction between receptor and enzyme is diffusion controlled.

The inverse relationship between the rate of a diffusion-controlled bimolecular reaction and the viscosity of the medium is well established[15,16]. When the bimolecular reaction occurs in two dimensions the relationship is[17]:

$$k_1 = \frac{k_\mathrm{obs}}{[\mathrm{R_T}]} = \frac{N_\mathrm{A}\cdot 4\pi \cdot D_\mathrm{R,E}}{\ln\left[\left(\frac{\pi}{4}[\mathrm{R_T}]N_\mathrm{A}\right)^{1/2}\cdot\frac{1}{a}\right]} \qquad (6)$$

where k_1 is the bimolecular rate constant (equation (1)), k_obs the measured pseudo first-order rate of enzyme activation (equations (3) and (4)), $D_\mathrm{R,E}$ relative diffusion coefficient, $[\mathrm{R_T}]$ total receptor concentration, N_A Avogadro's number and a the

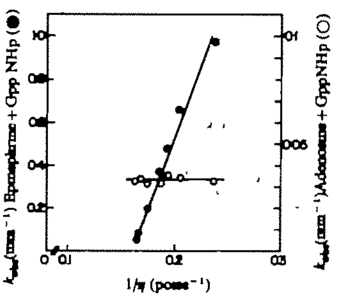

Fig. 1 The dependence of the rate constant of adenylate cyclase activation by *l*-adrenaline and by adenosine on membrane fluidity. Adenosine- and *l*-adrenaline-induced first-order processes of adenylate cyclase activation were measured as a function of increasing membrane fluidity. The kinetics of enzyme activation were determined and analysed as described in the text, using equation (3) to fit the hormone-induced activation curves. The fluidity of the membrane was increased progressively by treating the membrane with *cis*-vaccenic acid as described earlier[13] The fluidity of the membranes was measured by fluorescence polarisation using DPH as a fluorescence probe[14] (●), Dependence of k_{obs} for adrenaline activation on membrane fluidity; (○), dependence of k_{obs} for adenosine activation on membrane fluidity.

length of receptor to enzyme interaction domain. As the diffusion coefficient is inversely proportional the viscosity of the medium (Stokes'–Einstein relation) one can rewrite equation (6) as:

$$k_{obs} = \frac{N_A \cdot 4\pi[R_T]}{\ln\left[\left(\frac{\pi}{4}[R_T]N_A\right)^{1/2} \cdot \frac{1}{a}\right]} \times \frac{C}{\eta} \qquad (7)$$

where C is a constant and η the viscosity of the medium. From the slope of the plot of k_{obs} against $1/\eta$ the value of C can be calculated. Knowing η one can calculate the value of $D_{R,E}$.

Using the relationship between the bimolecular rate constant k_1 and the viscosity of the medium in a diffusion-controlled reaction in two dimensional space[17], one can calculate that the diffusion coefficient describing the relative motion of the receptor and the enzyme is in the range of 4.0×10^{-11} to 9×10^{-10} cm^2 s^{-1} at 25 °C. The value of k_1 can be obtained from k_{obs} ($k_1 \sim 1 \times 10^{13}$ min^{-1} (mol cm^{-2})$^{-1}$ (equation (4)) as $[R_T]$ is known for turkey erythrocytes[18,19]. This range of values for the diffusion coefficient is typical for mobile membrane receptors[20,21]. These values range from 2×10^{-11} to 4×10^{-9} cm^2 s^{-1}. The independence of the adenosine-induced rate of enzyme activation on membrane fluidity (Fig. 1) confirms previous biochemical studies[11,12] which showed that this receptor is permanently coupled to the cyclase.

The validity of the fluorescence depolarisation technique as a measure for membrane viscosity has been questioned[22]. It seems that even if the method does not yield the absolute value of the membrane viscosity these values are directly proportional to the viscosity. The fact that the data presented in this letter confirms the biochemical studies supports the view that the fluorescence polarisation technique is a powerful tool for measuring membrane viscosity. Furthermore, viscosity data which are derived from the rotation of small lipid-soluble molecules in the plane of the membrane approximate viscosity measured in other ways, for example by following diffusion of membrane proteins[23].

Membrane fluidity also affects the catalytic unit directly, as the maximal activity of the enzyme increases as a function of membrane fluidity. From Fig. 2 it is apparent that the adenosine-dependent activity, the adrenaline-dependent activity and the NaF-stimulated activity increase as a function of membrane fluidity in a similar way. Similar effects of membrane viscosity on the adenylate cyclase activity were recently reported[24,25]. The specific activity of other membrane bound systems, such as Ca^{2+}-ATPase[26], (Na^+, K^+)ATPase[27] and the β-galactoside transport system[28] were also found to depend on the membrane fluidity.

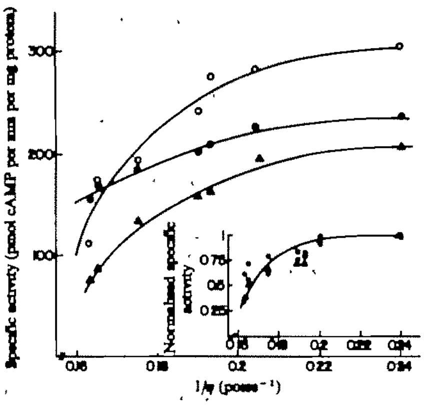

Fig. 2 The dependence of adenylate cyclase specific activity on membrane fluidity. The specific activity of adenylate cyclase as a function of membrane fluidity was measured in the presence of either 0.10 mM *l*-adrenaline plus 0.1 mM GppNHp (○), 0.1 mM adenosine plus 1.0×10^{-4} GppNHp (▲), or in the presence of 10 mM NaF (●). The assay mixture also contained 50 mM Tris-HCl, 6 mM $MgCl_2$, and 1 mM EDTA, pH 7.4, as described earlier[29]. Inset: The maximal specific activity for each mode of activation was taken as 1.0 and the relative increase in specific activity as a function of membrane fluidity was plotted. In this way one can compare the dependence of the three modes of activation with each other.

The dependence of the rate of adenylate cyclase activation by a hormone receptor on membrane fluidity can be adopted as a general diagnostic test for investigating coupling between a hormone receptor and adenylate cyclase. This approach can be extended to other membrane processes which involve the interaction between a receptor and a catalytic moiety, other than cyclase, such as an ionophore.

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Monoclonal antibody to a human histocompatibility alloantigen, HLA-A2

ANTIGENS of the HLA-A, -B, -C and -DR series have been defined mainly using sera from multiparous women[1], which generally are comparatively weak, serologically complex and available in only limited quantities. Furthermore, large screening programmes are required to find usable sera, especially for rare specificities. Although antisera to HLA determinants can be produced in high titres by immunising animals of various species with purified HLA-A, -B, -C, -D antigens[2–5], they usually require extensive absorption to reveal polymorphic specificity and so have not so far been used as routine tissue-typing reagents. The development of techniques for producing hybrid cell lines secreting monoclonal antibodies of defined specificity[6,7], provides a way of circumventing the problems of generating human polymorphic antisera by heteroimmunisation and has already been used to produce a monoclonal antibody against the human blood group A determinant[8]. Here we describe the production of a cell line secreting an anti-HLA-A2 antibody.

Female BALB/c mice were immunised subcutaneously with 20 µg of purified papain-solubilised HLA-A2 antigen[9] in complete Freund's adjuvant. After 1 month an equivalent boost in incomplete Freund's adjuvent was given and 2 weeks later the mice bled and their sera tested for anti-HLA-A, -B, -C antibodies. Serum titres of between 1 in 1,000 and 1 in 5,000 as measured by an indirect binding assay[10] on human B-cell lines expressing HLA-A, -B, -C antigens were observed. The titres against the B-cell line Daudi, which does not express HLA-A, -B, -C or the associated β_2 microglobulin (β_2m) antigens[11] were less than 50 and not significantly different from that obtained with pre-immune serum. No specificity for HLA-A2 could be detected by this method. The three mice with the highest serum titres were boosted intravenously with 10 µg of HLA-A2 antigen in saline, killed 5 days later, their spleens removed and the spleen cells fused with the mouse myeloma cell line P3-NSL/1-Ag4-1 (refs 6, 7, 12). After fusion the cells were divided between 192 two-ml culture wells. Visible colonies of hybrid cells were observed in all wells after 2 weeks and the culture supernatants were then tested for binding activity against an HLA-A2 positive B-cell line (Bri 8). Positive activity was seen in 109/192 supernatants, and the cultures from 39 that gave greater than 6 times the background were grown and their supernatants tested against Bri 8 and Daudi cell lines. This distinguished anti-HLA-A, -B, -C or β_2m antibodies from those directed against other cell surface components. Of the 39 cultures 38 showed no reaction with Daudi cells, thus indicating the high specificity of this procedure for generating antibodies to HLA-A, -B, -C or β_2m antigens. On continued growth, 14 cultures retained strong activity and were selected for further study. The binding activity of all 14 supernatants was fully inhibited by pre-incubation with purified HLA-A2 antigen, but only one of them could be inhibited by pure β_2m. Thus, the majority of the antibodies produced show specificity for the HLA-A, -B, -C chain, in contrast to one report suggesting a predominance of anti β_2m antibodies following immunisation with whole cells[13].

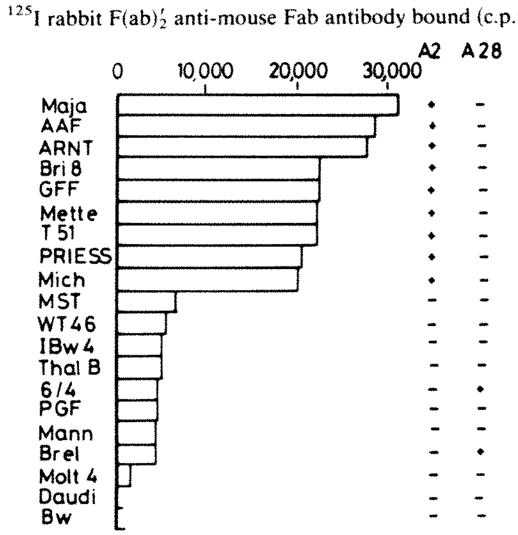

Fig. 1 Specificity of PA2.1 antibody for cell lines expressing HLA-A2 as measured by indirect trace binding assay. 10^6 cells were incubated with 50 µl of PA2.1 culture supernatant for 1 h at 4 °C, washed three times and then incubated with ^{125}I rabbit F(ab)$'_2$ anti-mouse Fab antibody, 300,000 c.p.m. for 1 h at 4 °C. The cells were washed four times and then counted for radioactivity. BW is a mouse thymoma cell line, Molt 4 a human T-cell line, all others are human B-cell lines[8].

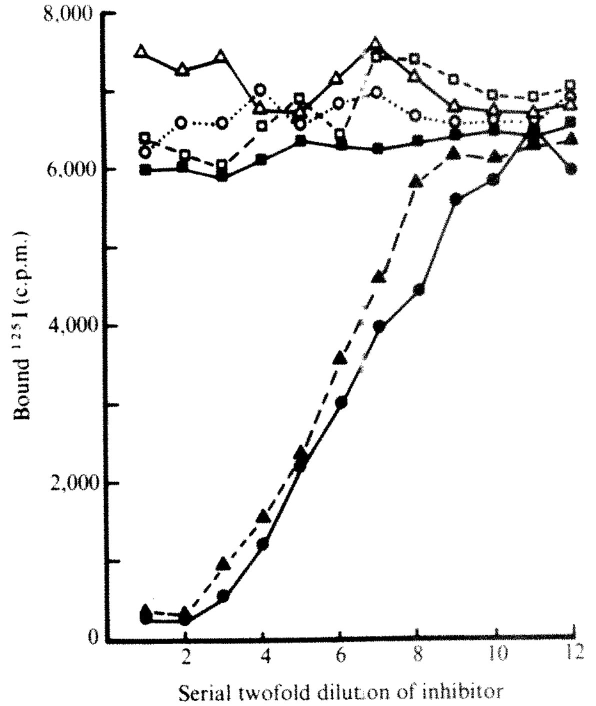

Fig. 2 Inhibition of PA2.1 antibody by purified papain-solubilised HLA-A, -B antigens with different antigenic specificity. HLA-A, -B antigen preparations (200 µg ml^{-1}) were serially diluted and 50 µl preincubated with 5 µl of PA2.1 supernatant for 1 h at 20 °C. Residual PA2.1 binding activity was then measured as for Fig. 1 with Bri 8 as the target cell (see refs 9 and 21). ●, HLA-A2 from J. Y. cells (homozygous *A2, B7*); ▲, HLA-A2 from 231 cells (homozygous *A2, B27*); □, HLA-A28 from L.B. cells (homozygous *A28, B40*); △, HLA-B40 from LB cells, ○, a mixture of HLA-A3, 25, B12, 27 from IM-1 cells (A3, 25, B12, 27); ■, a mixture of HLA-B7, 12 from RPMI 4265 cells (A2; B7, 12).

Of the 14 supernatants 10 reacted equally with all the cells from a panel of lines expressing a range of HLA-A, -B, -C antigenic specificities and so probably recognise antigenic determinants common to all HLA-A, -B, -C antigens comparable to a monoclonal antibody (W6/32) which has previously been described[18]. Polymorphic reaction patterns[4] were observed in 4 supernatants. PA2.1 reacted only with cells typed as HLA-A2 and did not react with cells expressing the highly cross-reactive HLA-A28 antigen[14] (Fig. 1).

Supernatants PA2.2, PA2.3 and PA2.4 reacted with some HLA-A2-negative cells as well as all HLA-A2-positive cells. These antibodies probably recognise cross-reacting antigenic determinants which HLA-A2 shares with some other HLA-A, -B, -C antigens. Their detailed specificity is being analysed, but

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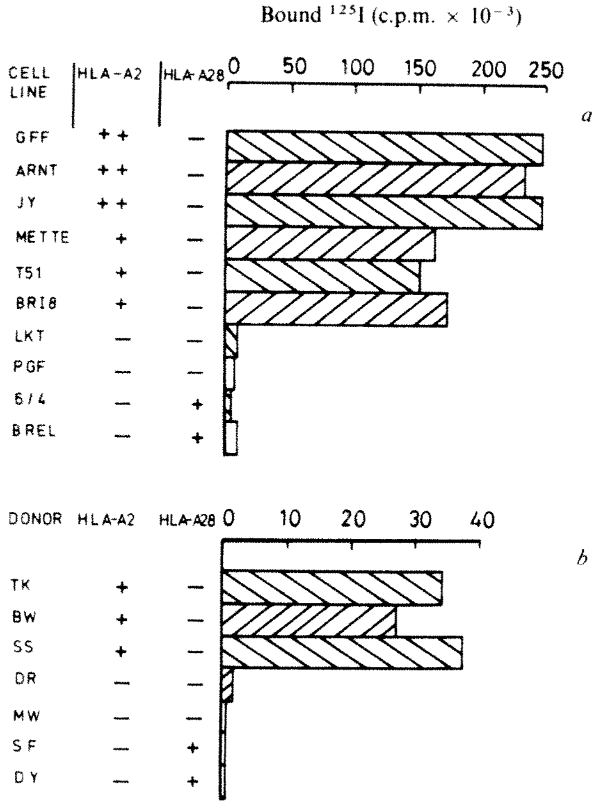

Fig. 3 Quantitation of HLA-A2 relative to total HLA-A, B, -C antigens on (*a*), B cell lines and (*b*), peripheral blood lymphocytes. The indirect binding of PA2.1 and W6/32 antibodies under saturating conditions of 1st antibody ($20\ \mu g\ ml^{-1}$) and 2nd antibody ($40\ \mu g\ ml^{-1}$) was measured on 5×10^5 cells in (*a*) and 2×10^6 cells in (*b*). The experimental procedure was as for Fig. 1 with an input of 3×10^6 counts per minute. To compare different cells within each group, the values plotted for the PA2.1 antibody binding have been normalised to a constant amount of radioactivity (representing total amount of HLA-A, -B, -C antigens) bound by the W6/32 antibody. This was the mean amount of radioactivity bound by the W6/32 antibody for all cells within a group and was 5×10^5 c.p.m. for B cell lines and 2.5×10^5 c.p.m. for peripheral blood lymphocytes. Cells are indicated as typed homozygous (++: based on family studies), heterozygous (+) and negative (−) for HLA-A2 and HLA-A28 by human alloantisera.

preliminary inhibition experiments with purified HLA-A, -B, -C antigens of different specificity indicate that supernatant PA2.2 probably reacts with a group including most HLA-A locus antigens.

Culture PA2.1 was cloned in soft agar[15] and one (PA2.1) of a number of colonies all with similar positive activity was arbitrarily selected for further study. The immunoglobulin secreted by PA2.1 was biosynthetically labelled with ^{14}C-lysine. Analysis by SDS-polyacrylamide gel electrophoresis, with other well characterised monoclonal antibodies as standards[16] showed that the PA2.1 antibody was an IgG molecule. It was not cytotoxic in complement-dependent antibody mediated cell lysis.

The specificity of the PA2.1 antibody for HLA-A2 was confirmed by inhibition with a variety of pure papain-solubilised HLA-A, -B, -C antigens (Fig. 2). Two preparations of HLA-A2 from different cell lines completely inhibited the PA2.1 antibody with similar specific activity, whereas four preparations representing eight other HLA-A, -B, -C antigenic specificities completely failed to inhibit. In particular, the PA2.1 antibody was not inhibited by the HLA-A28 preparation. It is unlikely that this was due to denaturation and loss of antigenic acitivity in the non-HLA-A2 antigen preparations, as all six fully inhibited the W6/32 monoclonal antibody, which recognises a determinant common to all HLA-A, -B, -C antigens that is dependent on the association of the HLA-A, -B, -C chain with β_2m and is easily inactivated by denaturation (data not shown).

HLA typing of B-cell lines often reveals patterns of serum cross reactions not usually observed with peripheral blood lymphocytes[17]. However, when PA2.1 antibody was tested by indirect binding against peripheral blood lymphocytes from a panel of 20 HLA-typed individuals it maintained its specificity for HLA-A2. Eight HLA-A2-positive individuals reacted strongly while 12 HLA-A2-negative individuals, including three who were HLA-A28 did not react (Fig. 3*b*). The same panel of peripheral lymphocytes was analysed in the fluorescence activated cell sorter after incubation with PA2.1 antibody and fluorescent labelled rabbit $F(ab)'_2$ anti mouse Fab antibody, using W3/25 monoclonal antibody with specificity for rat lymphocytes as control[18]. There was again complete concordance with HLA-A2, confirming the specificity of PA2.1 antibody for HLA-A2 and showing its potential use for cell separation. When the indirect binding assay is used with saturating amounts of antibody it is possible to quantitate the numbers of antigenic sites per cell[10]. By comparing the binding of PA2.1 and W6/32 antibodies, the proportion of the total HLA-A, -B, -C antigen which is HLA-A2 can also be estimated. As shown in Fig. 3*a*, cell lines homozygous and heterozygous for HLA-A2 could readily be distinguished. On this basis HLA-A2 accounted for 40–50% of the total HLA-A, -B, -C in homozygous cells and 30–35% of the total in heterozygous cells. Peripheral lymphocytes (Fig. 3*b*) had, per cell, about one eighth of the amount of HLA-A, -B, -C antigens found on B-cell lines as measured by the W6/32 antibody, although within each cell type the expression of HLA-A, -B, -C antigens varied by a factor of about two. In contrast to the B-cell lines, HLA-A2 accounts for only 12–15% of the total HLA-A, -B, -C expressed as W6/32 reactivity on the peripheral lymphocytes of individuals heterozygous for HLA-A2. This difference in apparent relative expression of HLA-A2 between EBV-transformed B-cell lines and a predominantly T-cell population of normal lymphocytes is being investigated further.

Xenogeneic immunisation between distantly related species, such as mouse and man, does not, in general, readily lead to the production of allospecific antibodies, although recently Lampson *et al.*[19] have produced limited quantities of anti-HLA-A2 antibodies using a mouse spleen cell culture system. We have shown, however, that it is possible by immunisation of mice with pure HLA antigen preparations to produce stable hybrid cell lines secreting mouse monoclonal antibodies directed at human polymorphic histocompatibility antigenic determinants. In addition to providing tissue typing reagents, these antibodies will be invaluable for the purification, separation and immunochemical analysis of HLA-A, -B, -C, -D molecules. We are, for example, now using the PA2.1 antibody to determine the antigenic structure of HLA-A2 and compare it with HLA-A28. Serological analysis of cross-reacting monoclonal antibodies such as PA2.2 may clarify the antigenic relationships between various HLA alleles and provide information on the evolution of this complex genetic system[20].

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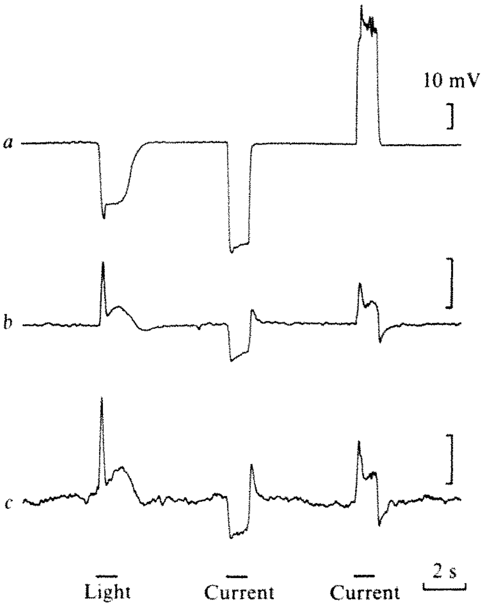
Effect of polarisation of horizontal cells on the on-centre bipolar cell of carp retina

THERE are two types of bipolar cell in the vertebrate retina, the on-centre and the off-centre cells[1,2]. The on-centre bipolar cells respond with depolarisation to the light spot in the centre of the receptive field and with hyperpolarisation to surround illumination. It is generally assumed that the effect of surround illumination is mediated by horizontal cells, as negative feedback from horizontal cells to photoreceptors has been demonstrated in the turtle retina[3]. Here we have attempted to clarify the role of horizontal cells in surround antagonism by hyperpolarising horizontal cells by applying extrinsic current in the retina of the carp, *Cyprinus carpio*. The response of the bipolar cells was similar to that elicited by surround illumination. However, the ionic mechanisms differed depending on the morphological type[4] of horizontal cell. An analysis of these mechanisms has led us to conclude that the external horizontal cells receiving inputs exclusively from cones modify the cone–bipolar transmission and the intermediate horizontal cells receiving inputs exclusively from rods modify the rod–bipolar transmission.

Fig. 1 Effect of polarisation of an intermediate horizontal cell on a bipolar cell. *a*, Potential changes of the intermediate horizontal cell; *b*, responses of the bipolar cell recorded simultaneously with *a*; *c*, responses of the same bipolar cell during hyperpolarisation of its membrane by steady current of 2.3 nA. In each trace the response to diffuse white light was tested first (left), then the horizontal cell was polarised in the dark to both hyperpolarising (middle) and depolarising (right) directions by extrinsic current through one barrel of a double-barrelled electrode. Timing of light and current stimuli is indicated below. The current used to polarise the horizontal cell was 10 nA. The coupling resistance of the double-barrelled electrode was 0.9 MΩ when measured in Ringer solution. The voltage drop across the coupling resistance was not compensated in these experiments. Calibration of 10 mV is shown at the end of each trace.



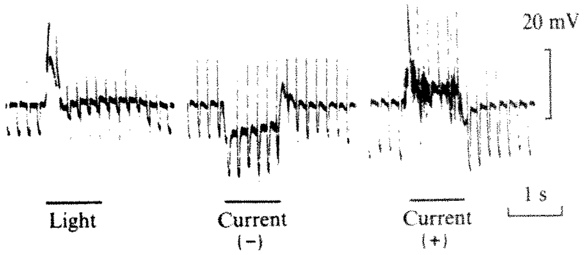


Fig. 2 Bridge records of bipolar cell responses to light (left) and to hyperpolarisation (middle) and depolarisation (right) of an intermediate horizontal cell by extrinsic current. Negative current pulses of 2.4 nA and of 50 ms duration were applied into the bipolar cell at a frequency of 5 pulses s^{-1} through the bridge circuit. The bridge was adjusted so that the decrease in the membrane resistance was indicated by a decrease in the height of pulses deflecting downward. The 20-mV change in the pulse height corresponds to 8.3-MΩ change in the membrane resistance. The response to light is accompanied by a resistance decrease. The hyperpolarising response to polarisation of the horizontal cell is accompanied by an increase and the depolarising response by a decrease in the membrane resistance.

Cajal[4] has described three morphologically different types of horizontal cell in the teleost fish retina; external, intermediate and internal. Both morphological and physiological studies have shown that external horizontal cells receive inputs exclusively from cones and intermediate horizontal cells exclusively from rods[2,5,6]. Recent studies further revealed two sublayers of external horizontal cells[7,8]. The cells in the distal layer are not colour-coded and give the response known as the L-type. Those in the second layer are colour-coded and give the response known as the C-type. The internal horizontal cells of Cajal are now considered as the processes of external horizontal cells[9]. These different types of horizontal cells were polarised in the present experiments by extrinsic current and its effect on bipolar cells was studied. The bipolar cells studied in these experiments were on-centre cells, because they were most frequently recorded and their responses were relatively stable. The bipolar cells recorded were identified by staining with Procion yellow as the large bipolar cells of Cajal which are known to have connections with both rods and cones[5,10].

Single- and double-barrelled microelectrodes, which could be advanced independently, were set almost parallel with their tip distance within 50 μm on the horizontal plane and were inserted into the isolated inverted retina vertically from above. When the responses of a bipolar and a horizontal cell were recorded, by single- and double-barrelled electrodes respectively, their cell types were identified by the response to diffuse white light of about 0.4 lm cm^{-2} and by changing patterns and wavelengths of the light stimulus if necessary. After confirmation of the response types, an extrinsic current of the order of 10 nA was passed through one barrel of the double-barrelled electrode in the absence of light but at the same timing as the light stimulus. In order to examine the ionic mechanisms of bipolar cell responses elicited by polarisation of horizontal cells, the membrane potential of bipolar cells was also changed by a steady polarising current or a train of current pulses of the order of 2 nA through a bridge circuit built in a preamplifier (WPI M701R). The steady current was used to estimate the reversal potential and the pulse current to measure the membrane resistance changes.

An example of simultaneous recordings from an intermediate horizontal cell (*a*) and an on-centre bipolar cell (*b*) is shown in

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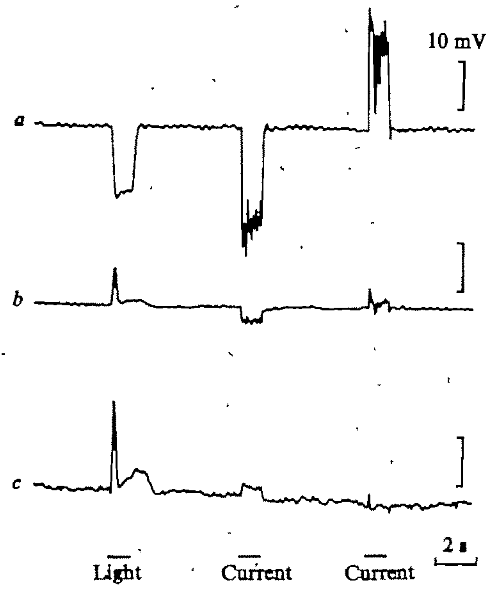
Fig. 1. The response of intermediate horizontal cells can be easily distinguished from that of external horizontal cells by its time course. The hyperpolarisation of intermediate horizontal cells by current elicited a hyperpolarising response in the on-centre cell and the depolarisation a depolarising response. When the bipolar cell was hyperpolarised by a steady extrinsic current, both hyperpolarising and depolarising responses elicited by the horizontal cell polarisation were augmented (*c*). The depolarisation of the bipolar cell resulted in a decrease in the response amplitude. The measurement of the membrane resistance changes revealed that the hyperpolarising response was accompanied by an increase and the depolarising response by a decrease in the membrane resistance (Fig. 2). It is inferred from these results that the effect of polarisation of intermediate horizontal cells is mediated by ionic channels such as sodium channels which have a positive reversal potential. These channels are made more permeable by depolarisation and less permeable by hyperpolarisation of intermediate horizontal cells.

An example of simultaneous recordings from an L-type external horizontal cell (*a*) and an on-centre bipolar cell (*b*) is shown in Fig. 3. The hyperpolarisation of external horizontal cells also elicited a hyperpolarising response in the on-centre cells and the depolarisation a depolarising response. These responses were made smaller or inverted by hyperpolarisation of the bipolar cell membrane by steady extrinsic current (*c*). They were augmented by depolarisation of the bipolar cell. The measurement of the membrane resistance changes revealed that the hyperpolarising response was accompanied by a decrease and the depolarising response by an increase in the membrane resistance (Fig. 4). It seems therefore that the effect of polarisation of external horizontal cells is mediated by ionic channels which have a more negative reversal potential than the membrane potential in the dark like potassium and/or chloride channels, commonly observed in the inhibitory synapses. These channels are made more permeable by hyperpolarisation and less permeable by depolarisation of the external horizontal cells.

Studies on the synaptic transmission from photoreceptors to on-centre bipolar cells indicate that rod signals are mediated by a permeability increase of subsynaptic sodium channels in the light and cone signals by a permeability decrease of subsynaptic potassium and/or chloride channels[11]. Our results are consistent with these data if we assume that intermediate horizontal cells not only receive exclusive inputs from rods and selectively affect the rod–bipolar transmission in a way expected from the feedback hypothesis, but the external horizontal cells receive exclusive inputs from cones and selectively affect the cone–bipolar transmission.

Fig. 3 Effect of polarisation of an external horizontal cell on a bipolar cell. *a*, Potential changes of the external horizontal cell; *b*, responses of the bipolar cell recorded simultaneously with *a*; *c*, responses of the same bipolar cell during hyperpolarisation of its membrane by current. Recording conditions were the same as in Fig. 1 except that the current used to polarise the horizontal cell was 13 nA.



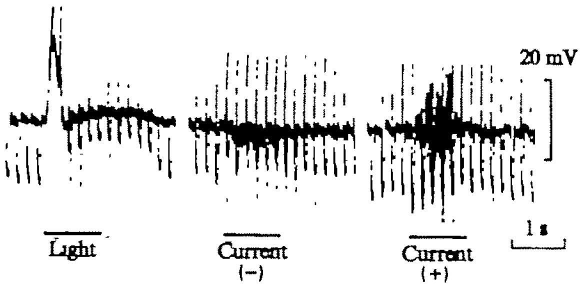


Fig. 4 Bridge records of bipolar cell responses to light (left) and to polarisation of an external horizontal cell by current (middle and right). Conditions of the bridge records were the same as in Fig. 2. The relatively large amount of current (20 nA) necessary to polarise the external horizontal cell generated an electrode noise, which was also picked up capacitatively in the bipolar cell records. Although the bridge records shown here are partly masked by such noise, it is seen that the hyperpolarising response to polarisation of the horizontal cell is accompanied by a decrease and the depolarising response by an increase in the membrane resistance.

The effect of polarisation of intermediate horizontal cells on the bipolar cells were clearly observed in all of the 11 pairs studied. On the other hand, the effect of polarisation of L-type external horizontal cells were observed in 5 out of more than 20 pairs studied. The effect was not prominent even in these 5 units. One reason for such results is that the input resistance of external horizontal cells is generally low which makes it difficult to polarise them sufficiently to affect the bipolar cells because of the restriction on the current flowing through the fine electrodes. It is also probable that the large bipolar cells receive a relatively small number of inputs from cones which mediate this effect.

The effect of polarisation of C-type horizontal cells on the on-centre bipolar cells was also studied in several pairs. But, so far we have been unable to detect any changes in the bipolar cells. It has been reported from morphological studies on goldfish retina that the C-type cells have direct connections with either blue or green cones but not with rods or red cones[12], and that a certain type of large bipolar cell has connections with rods and red cones but not with blue or green cones[13]. Although it is difficult to exclude the possibility that the current passed is not large enough to polarise the cell because of its low input resistance, the results probably indicate that the C-type cells do not affect the type of on-centre bipolar cells studied because they do not share the common photoreceptors.

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Change in synaptic channel gating during neuromuscular development

FORMATION of neuromuscular synapses involves accumulation of acetylcholine receptors (AChRs) in the postsynaptic membrane[1-5]. The ionic channels associated with synaptic AChRs differ from the AChR channels present in the surface membrane of non-innervated muscle fibres. In particular, the mean open time of synaptic channels is much shorter than that of non-synaptic channels[6-10]. The question therefore arises at which stage of neuromuscular development the gating properties of synaptic channels become similar to those observed in adult junctions. The experiments described here show a difference in the mean open time of channels in early neonatal and adult rat endplates and suggest that a change in gating behaviour takes place during postnatal development.

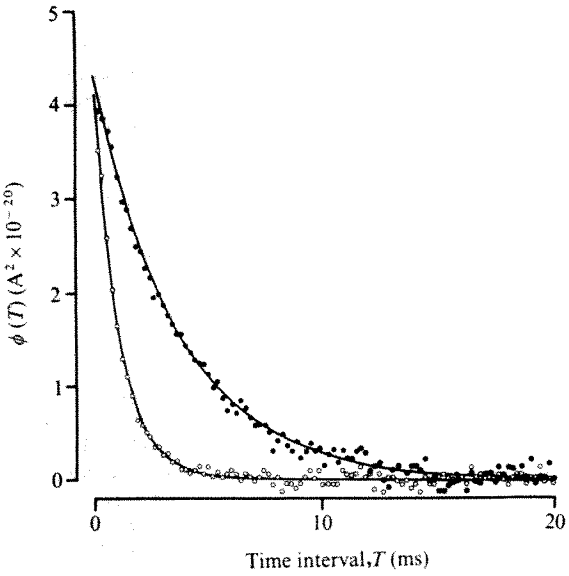

Fig. 1 Autocorrelation functions of ACh-induced current fluctuations recorded from voltage-clamped endplates of rat soleus muscle on postnatal days 8 (●) and 18 (○). Data points represent value of autocorrelation function $\phi(T)$ for different time intervals T. Mean ACh-induced currents were $I = 25$ nA and $I = 22$ nA, respectively. Membrane potential was clamped to −70 mV; temperature 22°C. Clamp currents before and during application of ACh were recorded on magnetic tape at low and high gain. The high gain record was bandpass filtered (1 Hz–2.5 kHz, 48 dB per octave roll off). High gain records were digitised at 200µs intervals and their a.c.f. calculated on a SAICOR auto-cross correlation analyser. Typically current samples of 6–12 s duration were analysed. a.c.fs of control and of ACh-evoked current samples were stored in a PDP 11/34 computer. The a.c.f. of the control sample was subtracted from the a.c.f. of the ACh-induced current sample. The resulting data points were displayed on an oscilloscope screen together with a machine-generated exponential (solid lines) corresponding to the expression $\phi(T) = \sigma^2 \cdot \exp(-T/\tau)$ where σ^2 represents the total variance of current fluctuations, and τ the a.c.f. decay time constant. For the a.c.fs shown, the curve-fitting yielded values of 3.9 ms (●) and 1.0 ms(○) for the decay time constant τ. Estimates of single channel conductances γ were obtained according to $\gamma = (\sigma^2/I)/(V - V_{rev})$ where V is the holding potential and V_{rev} the reversal potential (assumed to be 0 mV). The value of σ^2 is obtained from the extrapolated ordinal intercept of the fitted exponential. The single channel conductances were estimated as $\gamma = 25$ pS (day 8) and $\gamma = 27$ pS (day 18). All experiments were carried out in a solution containing (mM): NaCl, 140; KCl, 2; $MgCl_2$, 5; KH_2PO_4, 1; $NaHCO_3$, 12; pH 7, 2, oxygenated with 95% O_2 and 5% CO_2.

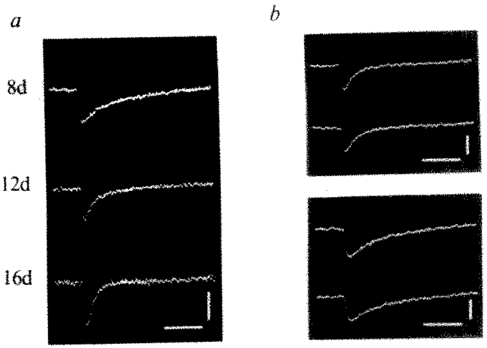

Fig. 2 Time course of m.e.p.cs at different stages of postnatal development. *a*, Change of soleus m.e.p.c. decay time course between postnatal days 8 and 16. Representative examples of single m.e.p.cs at different days after birth are shown. Note complex decay of m.e.p.cs on postnatal day 12. Recordings were made from soleus muscles of littermates. Membrane potential was clamped to −70 mV, 21–23°C. m.e.p.cs were recorded on magnetic tape and were replayed into a PDP-11/34 computer at a sampling rate of 20 kHz. Individual m.e.p.cs were displayed on an oscilloscope screen together with either one or the sum of two machine-generated exponentials which were fitted to the m.e.p.c. decay by eye. In the case of m.e.p.cs with doubly exponential decays one exponential was first fitted to the m.e.p.c. tail and subsequently another one to the difference between the data points and the extrapolated slower exponential. The mean $\bar{\tau}$ values in each fibre were on day 8: 4.6 ± 0.3 ms; on day 12: 1.1 ± 0.1 ms for the fast and 4.5 ± 0.3 ms for the slow component of m.e.p.c. decay; and on day 16: 1.2 ± 0.2 ms. Mean ± s.e.m., values from 10 individually measured m.e.p.cs were averaged in each fibre. Similar results were obtained with two other litters. Staining of endplate acetylcholinesterase activity by the Karnovsky method[17] did not show in the light microscope any obvious change in the staining intensity during this period. Calibration bars represent 4 ms and 2.5 nA. *b*, Recordings of m.e.p.cs on postnatal day 8 from neuromuscular junctions of omohyoideus (upper two traces) and soleus (lower two traces) muscle of the same rat. The average values of m.e.p.c. decay time constants were $\bar{\tau} = 1.3 \pm 0.1$ ms for the omohyoideus endplate and $\bar{\tau} = 4.3 \pm 0.2$ ms for the soleus endplate, averaging 10 individual m.e.p.cs in each experiment. Calibration bars represent 2 ms and 2 nA.

Experiments were performed at 20–23°C on isolated soleus and omohyoideus muscles taken from newborn rats between 1 and 18 d of age. Endplates were voltage clamped to −70 mV with a conventional two-microelectrode clamp. Acetylcholine (ACh) was released iontophoretically to induce steady endplate currents (e.p.cs) of 20–50 nA average amplitude. Channel mean open times were estimated from the autocorrelation function (a.c.f.) of fluctuations of the e.p.c. around its mean value. Assuming a simple two-state model of channel gating[6,11,12], the mean channel open time is given by the value of the decay time constant τ of the a.c.f.[7,12].

Figure 1 shows the a.c.fs of ACh-evoked e.p.c. fluctuations recorded from voltage clamped soleus endplates on postnatal days 8 and 18. The a.c.f. decay was singly exponential in both experiments but the decay time constants differed considerably. The value of τ on day 8 was 3.9 ms, which is about four times longer than the value of 1.0 ms found at the day 18 endplate. In seven experiments carried out before day 8, τ was 3.6 ± 0.2 ms (mean ± s.e.m.). This is comparable to the value found for channels in the extrasynaptic membrane of adult denervated[8] or early neonatal fibres (B.S. and H.R.B., unpublished) and for synapses formed *in vitro*[13]. Conversely, the mean channel open time observed in endplates on day 18 had an average value of $\tau = 1.0 \pm 0.1$ ms (four endplates), which is identical to that found in adult endplates[8,10,14]. From around postnatal day 8 to around day 17, however, the a.c.f. decay could be fitted adequately only by the sum of a fast and a slow exponential. Their average time constants were $\tau_{fast} = 1.0 \pm 0.1$ ms and $\tau_{slow} = 3.9 \pm 0.1$ ms (seven endplates). These are very similar to the time constants found at adult (≥18 d) and early neonatal (≤8 d) endplates, respectively. The amplitude of the fast component increased

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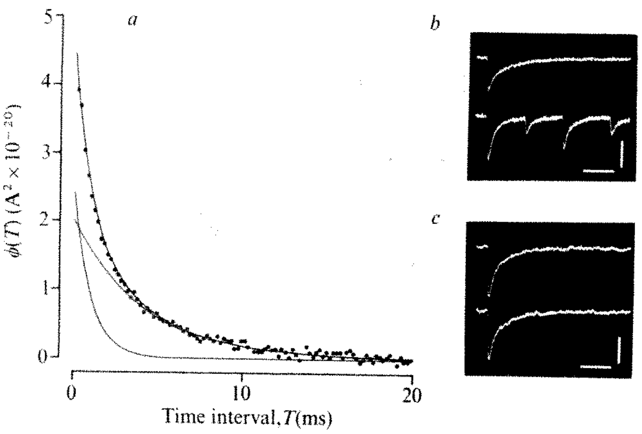

Fig. 3 Presence of two channel populations with different mean open times in neonatal omohyoideus endplate membrane. *a*, Autocorrelation function of endplate current fluctuations induced by iontophoretic ACh application on postnatal day 2. Data points represent value of the a.c.f. $\phi(T)$ for different time intervals T. The sum of two single exponentials, each of the form given in Fig. 1, was fitted to the data points (line drawn through data points). The two other lines represent the single exponentials. The time constants were $\tau_{\text{fast}} = 1.1$ ms for the fast and $\tau_{\text{slow}} = 3.9$ ms for the slow exponential. The ratio of their variances is $\sigma^2_{\text{fast}}/\sigma^2_{\text{slow}} = 1.18$. The slow exponential was first fitted to the tail of the a.c.f. decay, and subsequently the fast exponential was fitted to the difference between the data points and the extrapolated slow exponential. Membrane potential: −70 mV; 23°C. Mean ACh-induced endplate current was 21 nA. *b*, Recordings of m.e.p.cs on the same fibre as in *a*. m.e.p.c. decay is split into fast and slow component. The decay time course can be fitted by the sum of two exponentials with the time constants $\bar{\tau}_{\text{fast}} = 1.2$ ms and $\bar{\tau}_{\text{slow}} = 4.4$ ms. Mean values from 20 individually measured m.e.p.cs. *c*, Focal extracellular recordings of m.e.p.cs on postnatal day 4 from a voltage-clamped fibre (−70 mV, 22 °C). m.e.p.c. decay shows clear splitting in two decay phases and can be fitted by the sum of two exponentials. The respective time constants are $\bar{\tau}_{\text{fast}} = 1.0$ ms and $\bar{\tau}_{\text{slow}} = 5.3$ ms. The calibration bars represent 5 ms and 1 nA for *b* and 5 ms and 0.5 mV for *c*.

gradually during this transition period, whereas the amplitude of the slow component decreased correspondingly suggesting that from postnatal day 8 onwards the number of endplate channels with a short mean open time increases up to postnatal day 18 when all channels have fast kinetics.

Mean channel open time of junctional AChR channels can also be estimated from the time course of miniature endplate currents (m.e.p.cs). The falling phase of an m.e.p.c. in adult endplates is a single exponential the time constant of which, $\bar{\tau}$, approximately equals the mean open time of ACh-activated channels[11,15]. Developmental changes in the mean open time of junctional AChR channels should therefore be reflected in the m.e.p.c. decay time course. This is illustrated in Fig. 2*a* which shows examples of m.e.p.cs recorded from soleus endplates at various times after birth. To postnatal day 8 the m.e.p.c. decay is singly exponential with a mean value for $\bar{\tau}$ of 4.7 ± 0.2 ms (five endplates). This is four to five times greater than the value reported for adult endplates[14]. From around day 8 to around day 17, m.e.p.cs decayed with two time constants. From day 18 onwards m.e.p.c. decays were singly exponential again but with a much shorter time constant than is found in early neonatal fibres, the average value of $\bar{\tau}$ being 1.2 ± 0.1 ms (four endplates). During the transition period the m.e.p.c. decay could be satisfactorily fitted by the sum of two exponentials. Their time constants were similar to those of m.e.p.c. decays observed in early neonatal and adult endplates, respectively. The fast component becomes relatively more pronounced with age. For example, comparing measurements at four endplates on days 9 and 17 the average amplitude ratio of the fast to the slow component increased from 0.34 to 3.4.

A similar change in mean channel open time was observed in neonatal omohyoideus muscle, but it occurred earlier in postnatal development than in the soleus muscle. This is illustrated in Fig. 2*b*, which shows m.e.p.c. recordings from endplates in the omohyoideus and soleus muscles of the same rat at day 8. m.e.p.cs of the omohyoideus muscle decayed much faster than m.e.p.cs recorded from the soleus endplate. Their $\bar{\tau}$ value is comparable to that of m.e.p.cs in adult fibres already at this age. To determine the onset of the transition in channel gating in omohyoideus muscle, m.e.p.cs were recorded on postnatal days 1 and 2. In three out of four endplates studied on day 1 and in all four fibres studied on day 2, m.e.p.cs with doubly exponential decays were observed (Fig. 3*b*, *c*). The time constant of the fast component was $\bar{\tau}_{\text{fast}} = 1.2 \pm 0.1$ ms (seven endplates) comparable to the $\bar{\tau}$ value of m.e.p.cs in adult fibres. The average ratio of the slow to fast time constant was 4.3. Similarly the a.c.f. of ACh-induced e.p.c. fluctuations is fitted best by a double exponential decay during the first postnatal week (Fig. 3*a*) with the average time constants $\tau_{\text{fast}} = 1.2 \pm 0.1$ ms and $\tau_{\text{slow}} = 4.6 \pm 0.2$ ms (four endplates). From day 8 onwards only a fast component was resolved in both a.c.f. and m.e.p.c. decays, their respective time constants being $\tau = 1.0 \pm 0.08$ ms (four endplates) and $\bar{\tau} = 1.3 \pm 0.1$ ms (six endplates). This shows that synaptic channels in omohyoideus muscle have fast kinetics characteristic of the adult endplate, earlier during development than do those of soleus endplates. The fact that m.e.p.c. decays showed a fast component on postnatal day 1 suggests that in omohyoideus muscle a considerable fraction of adult-type channels is already present at birth.

Assuming neuromuscular synaptogenesis in rat begins at day 15 of gestation in the omohyoideus and at day 17 in soleus muscle[16], the experiments would indicate that a conversion of channels from an 'embryonic' to the adult type occurs in omohyoideus muscle between 6 and 14 d and in soleus between 13 and 22 d after the initial signs of neuromuscular transmission. It might be asked what influence causes the change of synaptic channel gating properties at a particular stage of development and why there is a difference between the omohyoideus and the soleus muscle? Neck muscles become active before the muscles of the lower extremities[16] and it is possible that earlier usage of neck muscles, for example during suckling, might cause this difference. Alternatively, maturation of neuromuscular junctions might be slower in red than in white muscle fibres.

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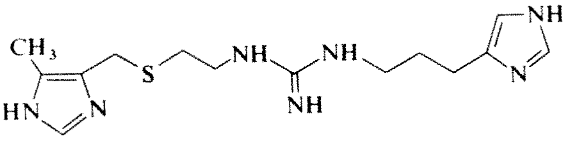
Impromidine (SK&F 92676) is a very potent and specific agonist for histamine H_2 receptors

PREVIOUSLY described selective agonists for histamine H_2 receptors are 4-methylhistamine[1], related congeners[2-6] and dimaprit[7]; these compounds are generally less potent than histamine. We have now found that *N*-[3-(imidazol-4-yl)-propyl]-*N'*-{2-[(5-methylimidazol-4-yl)methylthio]ethyl}-guanidine (impromidine, SK&F 92676 (I)) is extremely potent as an H_2-receptor agonist and briefly report its properties here.



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The action of impromidine was studied *in vitro* on two tissues known to contain histamine H_2 receptors[1], the guinea pig right atrium and the rat uterus; the results are summarised in Table 1. On the atrium, impromidine had 48 times the potency of histamine whereas on the uterus it was 9.3 times as potent. The maximum responses achievable by the two agonists were compared by construction of complete cumulative dose–response curves (Fig. 1). On the guinea pig atrium impromidine achieved the same maximum response as histamine but on the isolated rat uterus the maximum response relative to histamine was only 80%, clearly suggesting that the compound has a lower efficacy than histamine. Cimetidine[8] (a histamine H_2-receptor antagonist) competitively inhibited the action on the guinea pig atrium, giving a dose-related parallel displacement of the dose–response curve to the right without depressing the maximum response (see Table 1 legend). In the absence of propranolol, 100 μM cimetidine completely suppressed the response to impromidine (in concentrations of up to 1 μM) on the atrium, suggesting that it had no significant agonist interaction with adrenergic β receptors.

On the atropinised guinea pig isolated ileum, a tissue known to contain H_1 receptors, impromidine failed to exert an agonist effect at concentrations up to 10 mM. This indicates that it has <0.001% the agonist activity of histamine at H_1 receptors. In fact, impromidine competitively antagonised the agonist effect of histamine on the ileum ($pA_2 = 5.47$; Table 1). Impromidine also failed to stimulate contractions of the ileum in the absence of atropine, indicating that it is not a muscarinic agonist. Indeed, impromidine competitively antagonised the agonist action of carbachol on this preparation ($pA_2 = 4.89$; Table 1).

In vivo, impromidine was found to be a highly potent stimulant of gastric acid secretion (Table 1). Compared with histamine, using the technique of the lumen-perfused stomach of the anaesthetised, vagotomised rat[7,9], impromidine had 16.8 times the potency of histamine as a gastric acid secretagogue. The time course of the agonist response to impromidine after rapid intravenous injection was similar to that obtained to histamine. The infusion rate for near maximal acid secretion was 0.015 μmol per kg per min (compared with histamine, 0.25 μmol per kg per min). In the anaesthetised cat, impromidine was ~27 times as potent as histamine in stimulating gastric acid secretion, based on a comparison of the infusion rates producing 50% of the maximal response. As found with dimaprit[7], the maximal response seemed to be greater than that

Fig. 1 Isolated guinea pig right atrium; cumulative dose–response curves to histamine (●) and SK&F 92676 (○). Each point is the mean of 10 observations ± s.e.m. 0.5 μM propranolol was present in the bathing fluid.

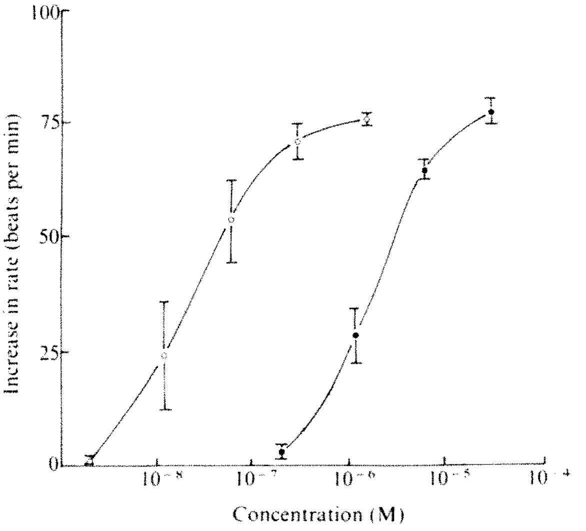

Table 1 Impromidine agonist activities, relative to histamine = 1

Preparation	Agonist activity mean (with 95% confidence limits)	% Maximum of histamine response (mean ± s.e.m.)	*n*
Guinea-pig ileum*	$<10^{-5}$		
Guinea-pig atrium†‡	48.1(37.6–60.1)	98.7 ± 3.6	10
Rat uterus†	9.3(5.7–15.2)	80.2 ± 2.6	22
Rat gastric acid secretion	16.8(11.3–27.1)	ND	16
Cat gastric acid secretion	~27	114 ± 18	5
Heidenhain pouch dog	~22	118 ± 11	5

SK&F 92676 was used as the trihydrochloride, a colourless crystalline solid, $C_{14}H_{23}N_7S$, 3HCl, of molecular weight 430.06 and melting point 198–200°C. It is very soluble in water and posesses three basic groups; a 3×10^{-3} molar solution (0.13% w/v) gives a *p*H of approximately 4.5. At this *p*H, or at *p*H 7.4, solutions are stable for at least 30 d at 20°C. For the *in vivo* studies, solutions were made up in normal saline. Rat gastric acid secretion data were obtained using lumen-perfused stomach of the anaesthetised, vagotomised rat[7,9] in a 2+2 Latin square assay. Cat gastric acid secretion was obtained from anaesthetised cats fitted with an acute gastric fistula and gastric juice was collected by drainage under gravity[7]. Dose–response curves to intravenous infusions of the agonist were constructed as described for the dog in Fig. 2. Conscious dogs were fitted with Heidenhain pouches which had been prepared 1–3 yr previously. Dose–response curves were constructed as described in Fig. 2. ND, not determined.

* Agonist assay on the ileum in the presence of 1 μM atropine[7]. Other actions: H_1-receptor antagonism of histamine, regression of log (DR–1) on log (concentration of SK&F 92676) ($n = 12$) had a slope of 0.95 ± 0.18 and gave $pA_2 = 5.47$ (95% confidence limits 5.16–5.74). Antagonism of carbachol, regression of log (DR–1) on log (concentration of SK&F 92676) ($n = 12$) had a slope of 0.95 ± 0.26 and gave $pA_2 = 4.89$ (95% limits 4.18–5.41).

† Comparative assays in the presence of 0.5 μM propranolol using a 2+2 Latin square design[7]. The ED_{50} values for SK&F 92676 were, respectively, 0.025 μM and 0.18 μM on atrium and uterus.

‡ Cimetidine $pA_2 = 6.53$ (6.09–6.98) ($n = 15$), not significantly different from the value against histamine[8] ($pA_2 = 6.10(6.04–6.17)$). The slope of the regression of log (DR–1) on log (concentration of cimetidine) = 0.81 ± 0.21, not significantly different from unity.

Table 2 Blood pressure responses to doses of impromidine maximal for gastric acid secretion

Species	Dose of SK&F 92676 (μmol kg^{-1} min^{-1})	Mean fall in blood pressure (mm Hg)	Mean increase in pulse pressure (mm Hg)
		(*n* = 4)	
Rat	0.015	27	ND
Cat	0.0032	27	20
Dog	0.0008	27	14

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to histamine, but the difference was not statistically significant. The infusion rate for a maximum response was 0.0032 μmol per kg per min. In conscious Heidenhain pouch dogs, impromidine had ~22 times the potency of histamine (Fig. 2). The maximum response to impromidine was significantly greater than that obtained to histamine ($P = 0.05–0.025$). Maximal secretion was obtained at an infusion rate of 0.0008 μmol per kg per min (compare with histamine 0.022 μmol per kg per min). In all species studied the secretory response to impromidine was inhibited by histamine H_2-receptor antagonists. In the Heidenhain pouch dog, an infusion of cimetidine at 10 μmol per kg per h produced a mean inhibition of 86% ($n = 6$) of maximal acid secretion; this is comparable with that obtained against histamine for the same dose of cimetidine.

Blood pressure and gastric acid secretion were measured simultaneously in the anaesthetised rat and cat preparations and also in the acutely fistulated anaesthetised dog. The results shown in Table 2 indicate that at doses maximal for the stimulation of acid secretion, impromidine, in contrast to histamine, produced only small changes in mean blood pressure and in pulse pressure. Impromidine has been shown to be a specific agonist at histamine H_2 receptors. The lack of marked effects on the cardiovascular system at a dose level maximal for acid secretion suggests that impromidine may be useful as a diagnostic agent in man for the estimation of the maximal secretory capacity of the stomach in patients with gastrointestinal disease. Preliminary studies are reported elsewhere[10,11].

Subacute repeated dose studies have been carried out in the rat and dog for up to 14 d at dose levels >10 times the dose required for maximal stimulation of gastric acid secretion. No toxic effects which might limit the use of impromidine were apparent from these studies (G. B. Leslie, unpublished observations). Full details will be reported elsewhere.

The most interesting feature of the pharmacology of impromidine is its high potency compared to histamine. From the pharmacological analysis it seems that this increased potency is the result of an increased affinity of the compound for H_2 receptors rather than an increase in efficacy (using Stephenson's nomenclature[12]). This is obvious for the uterus preparation where impromidine is not a full agonist, although more potent than histamine; it would seem that in comparison with histamine many more receptors have to be occupied by impromidine to achieve a response. The high affinity of impromidine relative to histamine should make this a useful agent for binding studies at H_2 receptors.

The chemical structure poses interesting questions in relation to the pharmacological activity. Impromidine is a guanidine derivative having two different imidazolylalkyl substituents (Table 3, R_1 and R_2 in II), and it is instructive to compare it with analogous guanidines (Table 3, III and IV). The guanidine III (SK&F 91486) is a weak partial agonist at H_2 receptors[13] and must have a low efficacy relative to histamine. Adding the substituent R_2 to structure III gives impromidine and results in a full agonist on the atrium and in a 1,000-fold increase in potency. The group R_2 is, however, a structural feature of H_2-receptor antagonists (for example, metiamide[14,15] and cimetidine[8]) and, furthermore, the analogous guanidine IV (SK&F 92408) is an antagonist showing no agonist activity[16], suggesting that R_2 is associated with affinity but not with efficacy. Thus, a chemical group which contributes affinity in antagonist structures apparently converts a weak partial agonist (Table 3, III) into an agent (impromidine) which has increased efficacy as well as increased affinity. This result has important implications in considering drug–receptor interaction.

Fig. 2 Dog gastric secretion; dose–response curves to intravenous histamine (●) and SK&F 92676 (○) in the conscious Heidenhain pouch dog. Each point is the mean of five experiments ± s.e.m. Dose–response curves to intravenous infusion of the agonist were constructed using a stepwise doubling increment in the dose, each dose level being infused for 1 h and the mean of the acid output in the last two 15-min samples being taken to calculate the dose–response curve.

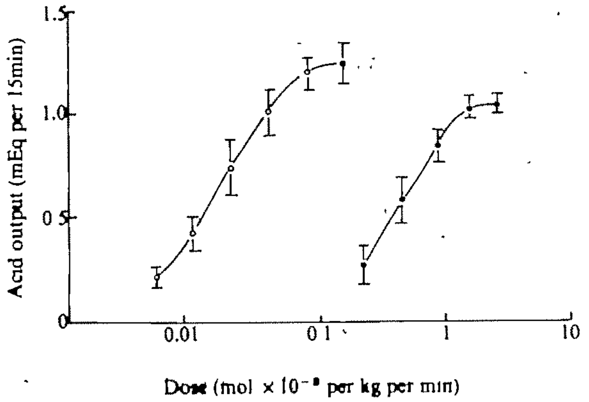
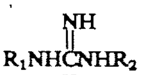
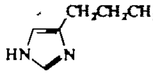
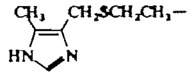
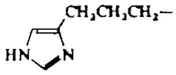
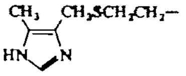


Table 3 Chemical structures of impromidine (SK&F 92676) and related guanidines



$$R_1NH\overset{\overset{NH}{\|}}{C}NHR_2$$

II

	R_1	R_2	H_2-receptor activity
I SK&F 92676			Potent agonist
III SK&F 91486		H	Weak partial agonist
IV SK&F 92408	CH_3		Antagonist

SK&F 91486 has 4% of the potency of histamine on the guinea pig isolated atrium but achieves only 70% of the maximum response relative to histamine; it competitively antagonises histamine ($pA_2 = 4.65$)[13]. These reported[13] results were obtained in the absence of propranolol; subsequent studies have shown that in the presence of propranolol, SK&F 91486 only attains 30.8±3.0% (mean±s.e.m.) of histamine's maximum response. On the rat isolated uterus (in the presence of propranolol) it has 6.5% of the potency of histamine and achieves only 27% of the maximal response[13]. SK&F 92408 competitively antagonises histamine[16], pA_2 4.80 (atrium), 5.26 (uterus).

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A new dopaminergic prodrug

THE clinical treatment of parkinsonism with the dopamine (DA) precursor L-dopa has certain drawbacks[1] and there are relatively few drugs which directly stimulate DA receptors (although there are many which block them, such as the neuroleptics). There is thus great industrial, academic and clinical interest in the development of new DA-receptor agonists[1]. Recently much interest has been given to 2-amino-6, 7-dihydroxytetrahydronaphthalene (ADTN)(Fig. 1), which is known from various *in vitro* and *in vivo* studies[2–10] to be a potent, selective and long-acting DA agonist. Two drawbacks to its more widespread study, however, are that the original synthesis was not easy, consisting of eight steps[11], and like DA, it does not readily pass the blood–brain barrier, so that it must be administered to animals intraventricularly. Although *in vivo* ADTN is not the most potent member of its group of 2-aminotetralin derivatives[12], it has the advantage of being structurally very similar to DA, but metabolically more stable. We have developed an improved, simplified synthesis of ADTN consisting of four steps[13] and we now describe how we have effected the passage of the drug into the brain. In addition to their practical and clinical implications, our results throw new light on the *in vivo* action of this drug.

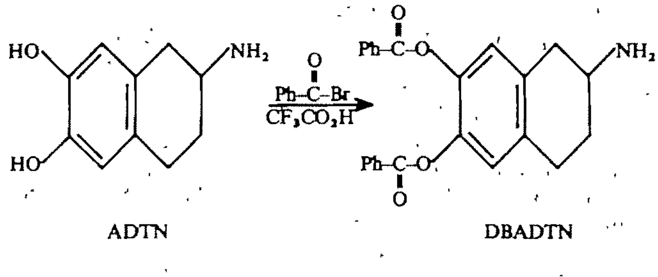

Fig. 1 The molecular weight of ADTN is 179 and that of DBADTN 387; thus, a dose of 50 mg per kg DBADTN is equivalent to 23 mg per kg ADTN.

As lipid-soluble drugs rapidly pass the blood–brain barrier, we synthesised a 'prodrug' from ADTN. A prodrug is a derivative that has certain desirable properties, for example, enhanced lipid solubility, which the parent molecule does not possess, and that can be converted back (by enzymes) into the active species within the body. The synthesis consisted of making a diester derivative of the catechol function (Fig. 1) which reduces the polarity of the parent molecule and makes it more lipid soluble. It was anticipated that after entering the brain the diester-prodrug would be converted back to ADTN by enzymatic hydrolysis. The dibenzoyl ester (DBADTN) was chosen as it was predicted on theoretical grounds that its rate of hydrolysis would be slower than that of the simple aliphatic esters[14], an important point if sufficient prodrug is to survive hydrolysis outside the brain, to pass into this organ. The diester was readily prepared in a yield of 74% by treating ADTN in trifluoroacetic acid with benzoyl bromide at room temperature (Fig. 1). The presence of trifluoroacetic acid ensures that only the catechol function is esterified and that the amino group is not affected. Analytical and spectral data were consistent with the proposed structure, and details will be published elsewhere.

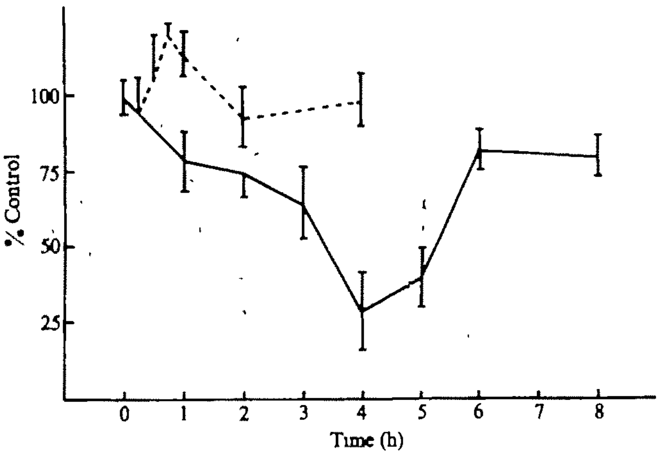

Fig. 2 Effect of DBADTN on HVA and DA concentrations in rat corpus striatum. Female Wistar rats (150–200 g) received 50 mg per kg DBADTN i.p. in a vehicle consisting of 30% ethanol, 30% water and 40% polyethylene glycol 400. HVA and DA were determined in rat corpus striatum using previously described methods[19,30]. Control values (mean ± s.e.m. (number of determinations)) for HVA were 0.52 ± 0.03 (27) µg per g and for DA, 5.68 ± 0.29 (5) µg per g wet weight. A statistical analysis consisting of an analysis of variance followed by the Newman–Keuls test[31] on the absolute values of HVA and DA showed that the decrease in HVA concentration at 3, 4 and 5 h was significantly different from control values at the 5%, 1% and 1% level, respectively. None of the changes in DA concentration were significant at the 5% level. Each point is the mean ± s.e.m. of 7–8 determinations for HVA and 4–8 for DA. – – –, DA; ——, HVA.

The idea that the prodrug would enter the brain and be hydrolysed to ADTN was tested by injecting 100 mg per kg DBADTN intraperitoneally (i.p.) into a female Wistar rat, killing the animal 45 min later, and rapidly dissecting the corpus striatum from the rest of the brain. The ADTN was isolated and derivatised with pentafluoropropionic anhydride using a modification of the method of Wilk and Stanley[15] for gas chromatography–mass spectrometry mass fragmentographic analysis. By monitoring the peaks at m/e 307 and 454 from the acylated derivative of ADTN, it was unequivocally shown that ADTN was present in the corpus striatum and the rest of the brain. DA agonists are known to decrease DA turnover in the corpus striatum as reflected by a lowering of the concentration of homovanillic acid (HVA)[16–18], one of the main metabolites.

The effect of 50 mg per kg DBADTN (equivalent to 23 mg per kg ADTN) on HVA levels in the corpus striatum of rats was studied over 8 h (Fig. 2). A 70% decrease of control levels was found after 4 h but by 6 h the HVA concentrations were almost normal. This slow onset of the maximum effect, in comparison with the rapid effect of apomorphine[19], is probably a result of the delayed concentration rise of ADTN in the brain brought about by enzymatic hydrolysis from the prodrug. Preliminary experiments indicate that lower doses of the prodrug also produce decreases in striatal HVA levels in a dose-related manner. As α-methyldopa is known to produce a very similar effect on HVA levels after the same time period[20] as well as lowering endogenous striatal DA levels, and as this may be brought about by its conversion to α-methyldopamine (which can be viewed as a ring-opened lower homologue of ADTN), the effect of DBADTN on striatal DA levels was also examined (Fig. 2). It was found however, that there was no statistically significant depletion of DA over a 4-h period. To ensure that the effect on

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HVA levels was due to ADTN produced in the brain and not to entry of ADTN produced by enzymatic hydrolysis outside the brain, 30 mg per kg of ADTN itself was injected i.p. into rats; however, after 4 h there was no significant lowering of HVA levels ($92\pm3\%(n=8)$ of controls) in the striatum.

It is known that the increase in DA turnover, as reflected in elevated HVA levels, which is caused by DA-receptor antagonists such as the neuroleptics, can be attenuated by DA agonists[17,21]. We were also able to demonstrate this with DBADTN in a dose-dependent manner; thus, the effects of 0.50, 0.25 and 0.05 mg per kg haloperidol could all be attenuated by administration of 50 mg per kg DBADTN (Fig. 3).

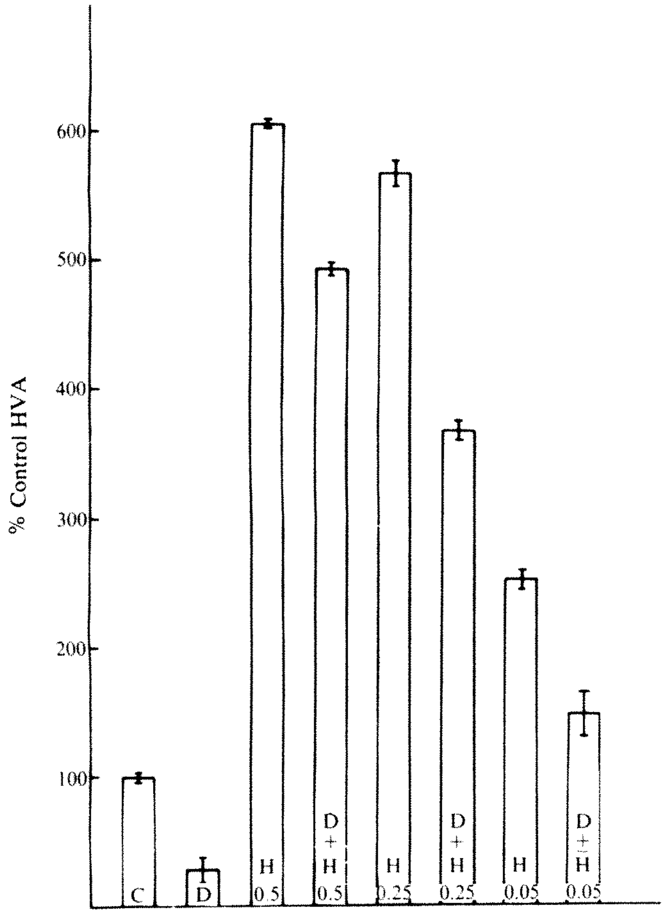

Fig. 3 Effects of DBADTN and various doses of haloperidol, alone and in combination, on HVA levels in rat corpus striatum. Female Wistar rats (150–200 g) received 50 mg per kg DBADTN (D) i.p. in the standard vehicle and 2.5 h after 0.50, 0.25 or 0.05 mg per kg haloperidol (H) i.p., the animals were then killed 1.5 h after the haloperidol injection. The HVA concentration in the corpus striatum was determined as previously described[19], control values (mean ± s.e.m. (number of determinations)) were 0.36 ± 0.01 (20) μg per g wet weight. Statistical analysis using Students t test on the HVA values in the experimental groups, haloperidol (H) against haloperidol (H) + DBADTN (D) showed that for the haloperidol doses of 0.5, 0.25, and 0.05 mg per kg the levels of significance were $P<0.01$ and <0.01 and <0.01, respectively. Each group is the mean ± s.e.m. for 7–8 determinations.

These results agree with the inhibition of the behavioural effects of ADTN by 0.5 mg per kg haloperidol[6]. It has now been shown that there are at least three DA receptors in the CNS, a postsynaptic and two 'presynaptic' receptors, one of which is probably located on the presynaptic nerve terminal, with the other near the DA cell body[22]. We have already presented *in vitro* evidence for an action of ADTN on the latter of these presynaptic DA receptors, that is, in the substantia nigra[23]. It was shown that in homogenates of this brain area, ADTN was as potent as DA in stimulating cyclic AMP production. It was therefore of interest to examine the effect of DBADTN (and hence ADTN) on the terminal presynaptic DA receptor using the γ-butyrolactone (GBL) model[22]. Administration of GBL to rats results in an inhibition of impulse flow in the dopaminergic presynaptic axons which causes a decrease in DA release coupled with a marked increase in its biosynthesis; this leads to a large rise in DA[22] and HVA[24] levels. The inhibition of these elevations by DA agonists is considered to be due to stimulation of presynaptic DA receptors on the axon terminal[22,24,25]. As shown in Fig. 4 the rise in HVA levels caused by GBL could be attenuated by pretreatment with DBADTN (compare G with D + G). This is compelling evidence for an action of ADTN on the presynaptic DA terminal[24]. Attempts to inhibit this effect of ADTN on HVA levels by blocking its uptake with benztropine[26] were without success, suggesting possibly that *in vivo* interaction with membrane presynaptic receptors is of more significance than is cytoplasmic accumulation of ADTN. *In vitro*, however, it has been shown[27] that benztropine does affect the ability of ADTN to interfere with DA turnover. Benztropine itself produced a lowering of HVA levels that was not statistically significant at the 5% level; Braestrup has also reported that it has no significant effect[28].

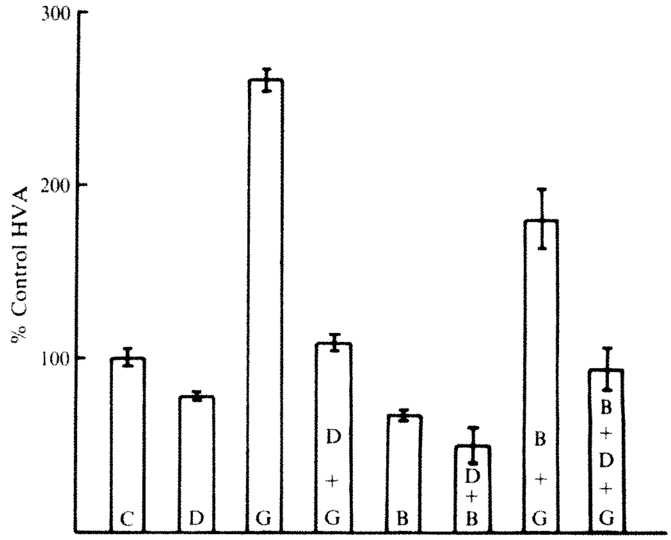

Fig. 4 Effect of DBADTN pretreatment on the GBL-induced rise in HVA levels in rat corpus striatum. Female Wistar rats (150–200 g) received 25 mg per kg benztropine[28] (B) i.p. and 10 min later 50 mg per kg DBADTN (D). 30 min after injection of D they were injected with 750 mg per kg γ-butyrolactone, GBL (G), and 90 min after this the animals were killed and HVA concentrations in the corpus striatum determined[19]. Control values were 0.54 ± 0.03 (14) μg per g wet weight (mean ± s.e.m.(number of determinations)). Statistical analysis (analysis of variance followed by the Newman–Keuls test[31]) on the HVA values showed that the differences between control (C) compared with G, and G compared with D + G were both significant at the 1% level. There was no significant difference at the 5% level between the groups D + G compared with B + D × G. Each group is the mean ± s.e.m. of 7–16 determinations.

We have demonstrated that it is possible to prepare a successful prodrug from a molecule that does not pass the blood–brain barrier. It should be stressed that the situation here is quite different from that occurring with the recently described esters of apomorphine[29] in that apomorphine itself can pass the blood–brain barrier and a reasonable amount of the free drug produced in the body by hydrolysis will probably pass into the brain. Second, we have shown that in addition to being able to interact with postsynaptic and nigral DA receptors, ADTN can also interact *in vivo* with DA receptors which are thought to be located on DA nerve terminals. We are now attempting to determine the concentration of ADTN in the corpus striatum and other brain areas and also to examine the effects of the prodrug on animal behaviour. The latter results may indicate whether or not the prodrug has any possible clinical potential.

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Alterations in β-adrenergic receptor binding during ethanol withdrawal

CHARACTERISTIC features of ethanol withdrawal, including tremor, agitation, seizures, tachycardia and increased blood pressure, frequently develop in chronic alcoholics 6–60 h after the discontinuation of long-term heavy ethanol consumption[1–4]. Similar symptoms are seen in hyperadrenergic states such as anxiety and thyrotoxicosis[5,6]. There is evidence to suggest that augmented sensitivity to catecholamines in thyrotoxicosis is due to the development of β-adrenergic receptor supersensitivity in cardiac and skeletal muscle cell surfaces[7–10]. Propranolol, a β-adrenergic blocker, has been shown to control some of the clinical signs of ethanol withdrawal, such as arrhythmia, hypertension and tremor[11–13]. In addition, propranolol significantly decreases urinary excretion of noradrenaline during ethanol withdrawal[14]. These findings have led to the suggestion that peripheral manifestations of increased adrenergic activity in ethanol withdrawal are secondary to increased sympathetic neural outflow and that this increase in central catecholaminergic outflow may be the result of increased β-adrenergic receptor sensitivity in the alcohol withdrawal state in man[14]. We report here attempts to determine whether increased central and peripheral β-adrenergic receptor binding could be demonstrated in rats during ethanol withdrawal.

Table 1 Effect of chronic ethanol treatment on specific binding of ^{3}H-DHA to β-adrenergic receptors in brain and heart particulate fractions

Time after termination of ethanol treatment (h)	Brain		Heart	
	Control	Ethanol-treated	Control	Ethanol-treated
0	37.7±0.57	29.1±0.96*	26.2±1.29	14.9±0.59*
24	33.5±2.81	36.1±3.39	28.2±1.37	32.7±1.05
48	26.9±2.36	47.2±4.00*	27.1±0.19	40.5±2.61*
72	31.6±1.66	52.8±4.19*	28.8±2.16	67.9±3.25*

Rats were pair-fed a nutritionally complete liquid diet containing either 6.5% (v/v) ethanol or an equicaloric amount of sucrose for 60 d by a previously described method[15]. Ethanol provided 36% of the total calories for the chronically treated rats (daily intake approximately 12–14 g per kg). The purity of ^{3}H-dihydroalprenolol (DHA) (NEN) was tested by thin-layer chromatography[16]. The procedures for the preparation of brain and heart particulate fractions and measurement of specific binding of ^{3}H-DHA to β-adrenergic receptors have been described previously[17–19]. The results are shown as fmol of DHA bound per mg protein and are the means (±s.e.m.) of 5 or 6 determinations (each in triplicate) from 5 or 6 rats in each group. The concentration of ^{3}H-DHA was 2 nM in each binding assay.

* Significantly different from control ($P<0.05$), by Student's *t*-test.

Specific binding of ^{3}H-dihydroalprenolol (DHA) to brain and heart particulate fractions obtained from sucrose- and ethanol-fed rats is shown in Table 1. The specific binding of ^{3}H-DHA in heart and brain particulate fractions decreased by 43% and 23% respectively in the ethanol-fed group as compared with the sucrose-fed control rats at zero hour of termination of chronic ethanol treatment. The high concentration of ethanol in rat blood at this time suggests that the decrease in ^{3}H-DHA binding might be due to interference by ethanol with the drug–receptor interaction. To test this possibility, specific binding of ^{3}H-DHA to heart and brain particulate fractions was measured in the absence or presence of ethanol in the binding assay medium. Ethanol (2 mg ml^{-1}) did not decrease binding of ^{3}H-DHA to heart or brain particulate fractions. In addition, 10 rats were divided into two groups; group I being treated intraperitoneally with ethanol, 2.5 g per kg body weight, administered as a 12.5% (w/v) solution in normal saline, and group II, which served as the control, receiving an equal volume of normal saline. Rats were killed 2 h after drug administration, and brain and heart particulate fractions were prepared as described in Table 1. Specific binding of ^{3}H-DHA to these preparations obtained from control and ethanol-treated rats were the same. These observations suggest that the decrease in specific binding of ^{3}H-DHA to the heart and brain particulate fractions immediately after termination of chronic ethanol treatment is not due to interference by ethanol with the drug–receptor interaction.

One day after discontinuation of chronic ethanol treatment, the specific binding of ^{3}H-DHA to heart and brain particulate fractions was similar in sucrose- and ethanol-fed rats. On the other hand, 48 h after cessation of ethanol administration, the specific binding of ^{3}H-DHA increased by 75% in brain and 49% in heart as compared with control preparations. Seventy-two hours after withdrawal from chronic alcohol administration the specific binding of ^{3}H-DHA was enhanced by 67% in brain and 136% in heart.

The increased binding of ^{3}H-DHA in rat brain particulate fractions during the withdrawal state following chronic ethanol treatment suggests changes in either the number or the apparent affinity of β-adrenergic receptors for ^{3}H-DHA. Therefore, the equilibrium dissociation constants and maximal number of binding sites for the β-adrenergic receptor were determined graphically (Fig. 1). Enhancement of the binding of ^{3}H-DHA to β-adrenergic receptors in rat brain particulate fractions 48 and

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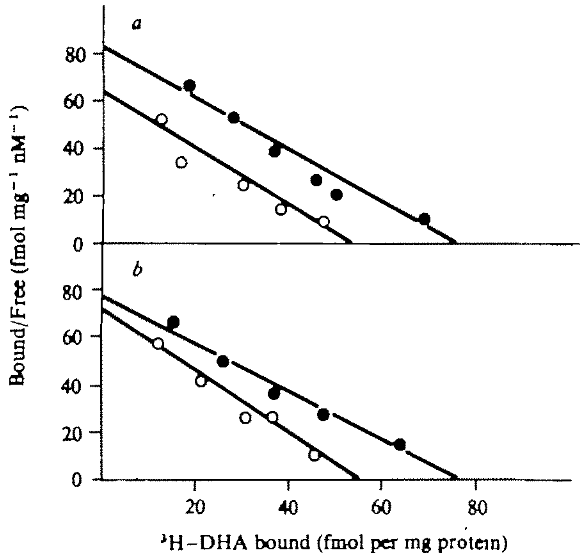

Fig. 1 Scatchard analysis of specific binding of ^{3}H-DHA in brain particulate fractions obtained from sucrose (○) and ethanol (●) fed rats at 48 h (*a*) and 72 h (*b*) after cessation of ethanol administration. The concentrations of ^{3}H-DHA used varied from 0.125 to 4 nM. Slopes and intercepts of the lines were determined by the method of least squares. The correlation coefficient, $r \geq 0.82$.

72 h after cessation of chronic ethanol intake appears to be due to an increase in the number of binding sites (Table 2).

Propranolol, a β-adrenergic receptor antagonist, has been shown to be of value in the management of withdrawal reactions of chronic alcoholism including augmented urinary excretion of noradrenaline[11-14]. These observations have led to the hypothesis that the peripheral effects of ethanol withdrawal may be a consequence of increased central β-adrenergic receptor sensitivity. The results described in the present communication provide experimental evidence in support of this hypothesis. In addition, present observations show that increased β-adrenergic receptor binding may occur both in the central nervous system and in peripheral tissues during ethanol withdrawal. Thus, the therapeutic efficacy of propranolol during ethanol withdrawal reactions may be mediated in part by its antagonism to the effects of increased central catecholaminergic outflow and in part by directly blocking hypersensitive β-adrenergic receptors in peripheral tissues.

The ethanol withdrawal state has been divided into two major components[1-4]. In mild reactions, the chief symptoms are sleeplessness and irritability, which appear within a few hours of withdrawal and disappear after 48 h. In severe reactions these symptoms are followed by tremulousness, hallucinosis, seizures, and arrhythmias which are most evident 2–3 d after ethanol withdrawal[1-4]. This suggests that the increase in β-adrenergic receptor sensitivity may contribute to the withdrawal reaction following the interruption of chronic ethanol intake.

Attenuated as well as increased accumulations of noradrenaline-stimulated cerebral cortical cyclic AMP have been reported with chronic ethanol treatment in rats and mice[20-22]. As noradrenaline may increase formation of cyclic AMP by interacting with either α-adrenergic or β-adrenergic receptors in brain, the results of French, Israel and their associates[20-22] fail to provide any conclusive evidence for a specific alteration of sensitivity of α-adrenergic or β-adrenergic receptors in chronic alcoholism.

The molecular mechanism for the increase in β-adrenergic receptor binding in rat heart and brain particulate fractions during the ethanol withdrawal state is not known. A variety of psychotropic drugs, such as antipsychotics[23], antidepressants[17] and amphetamine[19], have been shown to alter the sensitivity of dopaminergic and β-adrenergic receptors. Amphetamine and desipramine are known to affect noradrenaline transport at the catecholaminergic nerve endings[24,25]. The Na^+-dependent noradrenaline transport appears to be mediated through the activity of $(Na^+ + K^+)$-ATPase[26,27]. A marked activation in the number of $(Na^+ + K^+)$-ATPase molecules in several areas of brain and some peripheral organs has been observed during ethanol withdrawal in cats and rats[28-31]. It is suggested that this activation of $(Na^+ + K^+)$-ATPase may contribute to increased β-adrenergic receptor sensitivity in rat heart and brain following interruption of chronic ethanol intake.

Table 2 Effect of chronic ethanol treatment on the equilibrium dissociation constant (K_D) and maximal number of binding sites (B_{max}) of β-adrenergic receptors in rat brain

Time after termination of ethanol treatment (h)	Control B_{max} (fmol per mg protein)	Control K_D (nm)	Ethanol-treated B_{max} (fmol per mg protein)	Ethanol-treated K_D (nM)
0	62±6	0.71±0.06	45+7*	0.81±0.07
48	53±8	0.85±0.07	76±11*	0 91±0.06
72	55±9	0.77±0.05	76+9*	0.98±0.05

The specific binding of 5 concentrations of ^{3}H-DHA (0.125–4 nM) to β-adrenergic receptors in rat brain particulate fractions was assayed as described in Table 1. The equilibrium dissociation constants and maximal number of binding sites were estimated by Scatchard analysis, as described before[17-19]. Results shown are the mean (±s.e.m) of 5 determinations (each in triplicate) from 5 rats for each of the 5 concentrations of ^{3}H-DHA used

* Significantly different from control ($P < 0.05$), by Student's *t*-test.

The studies presented here suggest that increased β-adrenergic receptor binding in rat brain and peripheral tissues may contribute to the hyperadrenergic state of the withdrawal reaction of chronic alcoholism. These observations provide a rationale for the use of β-adrenergic antagonists in the management of the ethanol withdrawal reaction.

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Epidermal growth factor stimulates phosphorylation in membrane preparations *in vitro*

EPIDERMAL GROWTH FACTOR (EGF) forms a complex with plasma membrane receptors in intact cells that initiates a series of biochemical events resulting in increased cell growth *in vivo* and *in vitro*[1]. The interaction of EGF with membrane receptors has been demonstrated in crude membrane preparations[2], but no biochemical alteration of the membrane resulting from hormone binding has been detected. To clarify the molecular mechanisms regulating cell proliferation, specific biochemical reactions initiated by mitogens such as EGF need to be investigated in cell-free systems. As the human epidermoid carcinoma cell line A-431 has an extraordinarily high concentration of EGF receptors[3,4] (2–3 $\times 10^6$ receptors per cell), we have used a crude membrane preparation from these cells to look for an EGF-dependent alteration of membrane structure and/or function. We report here that (1) membranes may be prepared from A-431 cells that retain the ability to bind ^{125}I-labelled EGF in a specific manner, and (2) the binding of EGF to these membranes *in vitro* results in a marked stimulation of the phosphorylation of endogenous proteins in the presence of [γ-^{32}P]ATP.

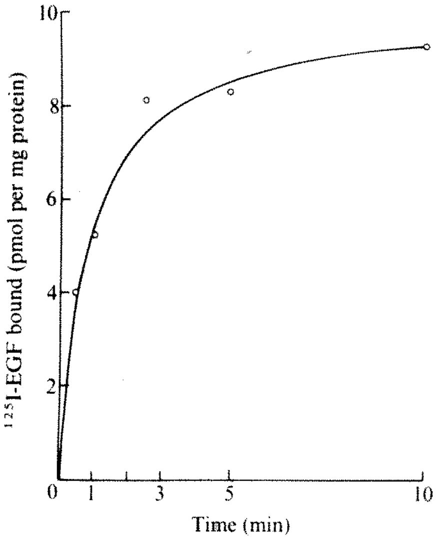

Fig. 1 Binding of ^{125}I-EGF at 0 °C to A-431 membranes. Membranes were prepared by the procedure described by Thom *et al.*[5] from A-431 tumour cells grown in Falcon culture dishes containing Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and gentamycin. ^{125}I-EGF was prepared as reported by Carpenter and Cohen[6]. The binding reaction was carried out in a 200-μl reaction volume containing 20 mM HEPES buffer, *p*H 7.4, 0.1% bovine serum albumin (BSA), 12 μg of A-431 membrane protein (measured by the Bradford procedure[7] using γ-globulin as a standard), and ^{125}I-EGF (3.3×10^{-9}M). Nonspecific binding was measured in co-lateral reaction tubes which contained a 1,000-fold molar excess of unlabelled EGF (3.3×10^{-6} M) and did not exceed 4% of the total binding. The amount of ^{125}I-EGF bound to the membranes was determined by rapidly filtering the reaction mixture on Millipore EGWP filters, washing each filter with a total of 8 ml of 20 mM HEPES, *p*H 7.4, containing 0.1% BSA. The amount of radioactivity retained on each filter was determined by counting in a Nuclear Chicago γ-spectrometer. Storage of A-431 membranes at −70 °C for 2–3 months did not appreciably decrease the binding activity.

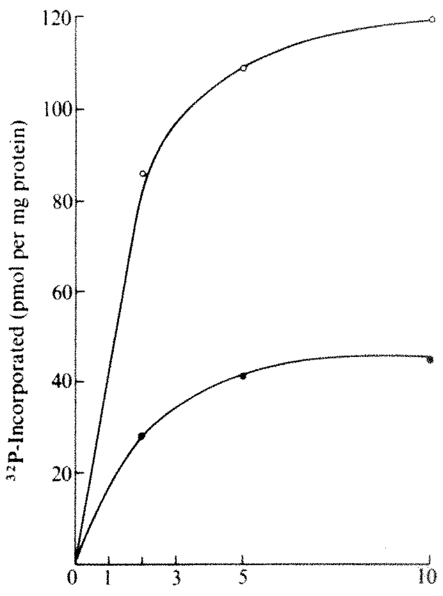

Fig. 2 Stimulation by EGF of the incorporation of ^{32}P-phosphate from [γ-^{32}P]ATP into cell membranes. The reaction mixtures contained A-431 membrane (27 μg protein), HEPES buffer (20 mM, *p*H 7.4), $MnCl_2$ (1 mM), [γ-^{32}P]ATP (15 μM, 8×10^5 c.p.m.), EGF (40 ng) and BSA (7.5 μg) in a final volume of 60 μl. The reaction tubes were placed on ice and preincubated for 10 min in the presence (○) or absence (●) of EGF. The reaction was initiated by the addition of labelled ATP and incubation at 0 °C was continued for the indicated times. The reaction was terminated by pipetting 50-μl aliquots on to squares (2 cm) of Whatman 3 MM filter paper and immediately dropping the paper into a beaker of cold 10% TCA containing 0.01 M sodium pyrophosphate. The filter papers were washed extensively with pyrophosphate-containing 10% TCA at room temperature, extracted with alcohol and ether, dried, and the radioactivity measured in a Nuclear Chicago gas-flow counter.

The specific binding of ^{125}I-labelled EGF at 0 °C to membranes prepared from A-431 tumour cells is shown in Fig. 1. In these conditions, the initial rate of binding is rapid (8 pmol of ^{125}I-EGF are bound per min per mg protein) and equilibrium is reached within 10 min. At equilibrium, approximately 18% of the hormone is membrane-bound at a specific binding activity of 9.0 pmol of EGF per mg protein. A-431 membranes, therefore, contain a concentration of EGF receptors comparable to the high levels detected in membranes prepared from human placenta[2].

Aliquots of the A-431 membrane preparation were used to determine whether the binding of EGF to membrane receptors was followed by a biochemical alteration of the membranes. As shown in Fig. 2, the addition of EGF to A-431 membranes produced a three- to fourfold stimulation of the phosphorylation of endogenous membrane proteins at 0 °C as judged by the transfer of ^{32}P-phosphate from γ-labelled ATP to membrane material. Figure 2 also shows that in the presence of EGF the initial rate of phosphorylation is increased from 16.7 to 50.0 pmol of ^{32}P-phosphate incorporated per min per mg protein. Membrane protein phosphorylation reached a maximal level by 10 min at 0 °C and a threefold higher level of ^{32}P-phosphate incorporation was achieved in the presence of EGF at all times examined.

We have partially characterised this EGF-stimulated reaction in isolated A-431 membranes and here are summarising our results, the details of which will be presented elsewhere. The ^{32}P incorporated into the membrane is trichloroacetic acid (TCA)-insoluble and only a small amount (20%) is removed by extractions with alcohol, ether and chloroform–methanol. Partial acid

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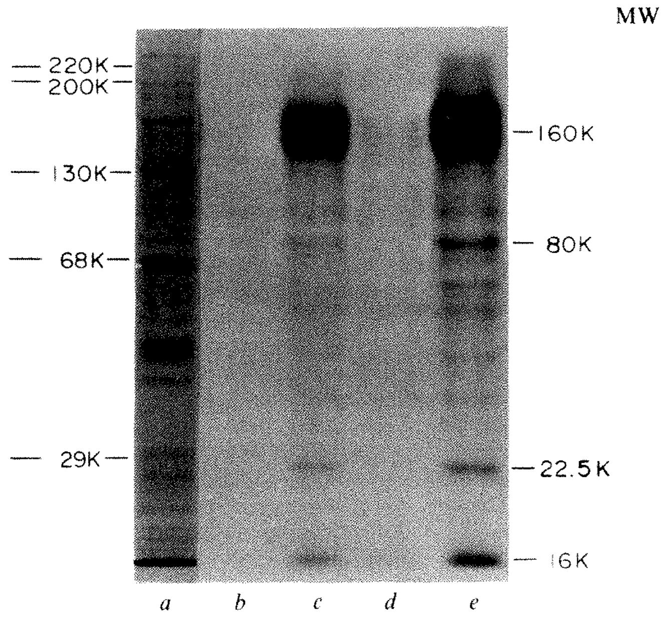

Fig. 3 Electrophoresis and autoradiography of A-431 membrane components incubated with [γ-^{32}P]ATP in the presence and absence of EGF. The phosphorylation of A-431 membranes was carried out at 0 °C in 40-µl reaction mixtures containing 30 µg of A-431 membranes, 20 mM HEPES, *p*H 7.4, 2 mM $MnCl_2$, 0.01% BSA and 15 µM [γ-^{32}P]ATP (20 Ci mmol^{-1}). EGF was added at a final concentration of 14×10^{-8} M immediately before the addition of ^{32}P-ATP, which initiated the reaction. The reaction was stopped after 1 or 5 min at 0 °C by the addition of 40 µl of Laemmli SDS sample buffer[8]. The mixture was boiled for 5 min and then subjected to SDS-gel electrophoresis (7.5% acrylamide) according to the method of Laemmli[8]. The gels were stained with Coomassie blue, vacuum dried and autoradiography carried out by exposing the gel to Kodak RP Royal X-O mat film for 2 d. Molecular weight (MW) standards used for calibration were erythrocyte spectrin, 240,000 and 220,000; skeletal muscle myosin, 200,000; β-galactosidase, 130,000; BSA, 68,000; carbonic anhydrase, 29,000. *a*, Coomassie blue stain of A-431 membrane components (30 µg of protein applied to the gel); *b–e*, autoradiography of A-431 membrane components phosphorylated in absence (*b*, *d*) and presence (*c*, *e*) of EGF for 1 min (*b*, *c*) or 5 min (*d*, *e*).

hydrolysis of the phosphorylated membranes and electrophoresis of the resultant mixture show that in both the control and EGF-dependent reaction the major phosphorylated product is phosphothreonine, indicating that the observed phosphorylations are directed mainly towards protein substrates. Both the endogenous membrane phosphorylation and the EGF-stimulated reaction depend on the presence of Mg^{2+} or Mn^{2+}; Ca^{2+} is ineffective. Maximal stimulation of membrane phosphorylation occurs in the presence of 3×10^{-8} M EGF. The presence of cyclic AMP or cyclic GMP (10^{-4}–10^{-6} M) has no discernible effect on the reaction. Both the basal and EGF-stimulated reaction are completely destroyed by heating the membrane for 10 min at 50 °C before the addition of [γ-^{32}P]ATP and EGF. As expected, the addition of unlabelled ATP to the reaction mixture diminishes the extent of phosphorylation. The specificity of the EGF-dependent reaction was examined by the addition of a wide variety of peptide hormones to the reaction mixture in place of EGF. We did not detect any effect of insulin, thyrotropin, follicle-stimulating hormone, growth hormone, glucagon, prolactin or luteinising hormone on the overall phosphorylation reaction. The addition of EGF to the A-431 membrane preparation seems to stimulate a protein kinase activity capable of using exogenous substrates as well as the endogenous membrane substrates. Several proteins, including histones and ribonuclease are phosphorylated when they are added to this system and their phosphorylation is stimulated two- to fourfold by the addition of EGF.

The membrane components which were phosphorylated *in vitro* in the presence and absence of EGF were analysed by SDS–polyacrylamide gel electrophoresis and autoradiography. The results shown in Fig. 3 indicate that during the first minute of incubation in the presence of EGF, membrane components having molecular weights of ~170,000 and ~150,000 are heavily phosphorylated. With continued incubation, additional membrane components are phosphorylated to a greater extent in the presence of EGF, most noticeably components with apparent MW of 80,000 and 22,500. The results also show that membrane components for which phosphorylation is increased in the presence of EGF seem to be phosphorylated in the absence of the growth factor, but to a lesser extent. Of course, these data do not show whether different sites on the same membrane proteins are phosphorylated in the presence and absence of EGF.

Thus, we have been able to demonstrate for the first time the modulation of a biochemical reaction in a cell-free membrane preparation as a consequence of the formation of EGF–receptor complexes. Following the binding of EGF, the capacity of these membranes to phosphorylate endogenous and exogenous proteins is markedly enhanced. The mechanism by which EGF enhances phosphorylation is not known; the receptor may possibly be a protein kinase, a regulatory subunit of a protein kinase or a regulator of phosphoprotein phosphatase. However, the observed net increase in phosphorylation may be an even more indirect consequence of the formation of EGF–receptor complexes. As neither the identity nor function of the phosphorylated membrane components is known, it is not possible to relate these findings to specific metabolic alterations, such as increased transport of nutrients and ions or induction of DNA synthesis, that are induced in intact cells by EGF. We have previously shown that after the binding of EGF to intact cells, this mitogen, probably as a hormone–receptor complex, is internalised by cytoplasmic vesicles[3,6]. It is not known, however, at what stage or stages in the binding and internalisation of EGF intracellular signals are generated which regulate cell growth. We suggest that the phosphorylation of membrane or membrane-associated components may be an initial event in the generation of these important signals. The effect of external stimuli or protein phosphorylation has been noted in many systems *in vivo*[9], and with insulin, by the use of sarcolemma-enriched membrane preparations *in vitro*[10].

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Tubulin microheterogeneity increases with rat brain maturation

MICROTUBULES are present in all eukaryotic cells and have been found to have a variety of structural and dynamic roles in cell shape, division, motility, transport and secretion[1]. In nervous tissue neurite outgrowth and axoplasmic transport are also thought to depend on microtubule integrity[2]. The microtubule subunit protein, tubulin, is a heterodimer composed of two polypeptides α and β (refs 3, 4). The α and β subunits show microheterogeneity and both have been resolved into two or three components[5–8]. The question therefore arises as to whether changes occur in the relative proportions of the multiple forms of tubulin upon assumption of different roles within the nerve cell. We show here that cytoplasmic rat brain tubulin, as resolved by isoelectric focusing, is highly heterogeneous. Moreover, tubulin microheterogeneity seems to be developmentally determined, increasing from seven to nine distinct components during early postnatal rat brain maturation.

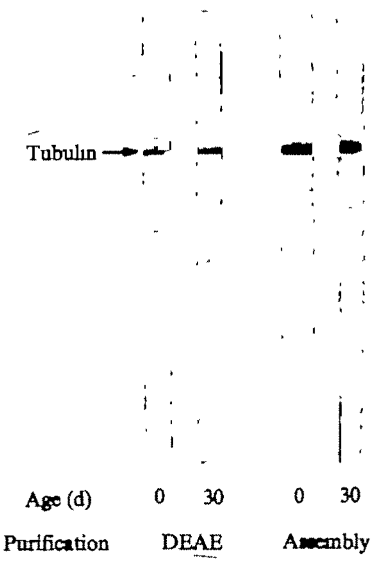

Fig. 1 SDS–polyacrylamide gel electropherogram of developing rat brain tubulin. Rat brain tubulin was prepared using DEAE-cellulose column chromatography following the procedure of Eipper[15] with slight modifications. Freshly obtained brains (2 g) from either newborn or 1-month-old rats were homogenised using a Dounce homogeniser, in 4 ml of 0.24 M sucrose, 2.5 mM $MgCl_2$ and 50 mM NaPPi, pH 7.0. The homogenate was centrifuged for 30 min at 30,000g and the supernatant obtained was loaded on to a DEAE-cellulose column (0.5×4 cm). After extensive washing with 0.1 M NaCl, 50 mM NaPPi, pH 7.0, and 2.5 mM $MgCl_2$, the tubulin peak was collected by elution with 0.26 M NaCl, 50 mM NaPPi, pH 7.0, and 2.5 mM $MgCl_2$, then dialysed against excess of H_2O and lyophylised. *In vitro* assembled tubulin was obtained by two cycles of polymerisation in the presence of 4 M glycerol as described by Shelanski *et al.*[16]. The purified tubulin preparations were dissolved in SDS sample buffer to yield a final concentration of 0.2 mg ml^{-1} protein, 10% glycerol, 5% 2-mercaptoethanol, 3% SDS, 0.0625 M Tris-HCl, pH 6.8, and 0.001% bromphenol blue and heated at 100 °C for 5 min. 10-µl aliquots were electrophoresed at 3 V cm^{-1} at 20 °C for 14–16 h on 0.75 mm thick polyacrylamide slab gels, containing 10–20% polyacrylamide gradient and 0.1% SDS[17,18]. The gels were stained with Coomassie brilliant blue.

However, extensive tubulin microheterogeneity is prominent in the brain, as tubulin from other organs is less heterogeneous.

Cytoplasmic tubulin from rat brain was identified by gel electrophoresis, its ability to undergo self-assembly, its purification by DEAE-cellulose column chromatography and

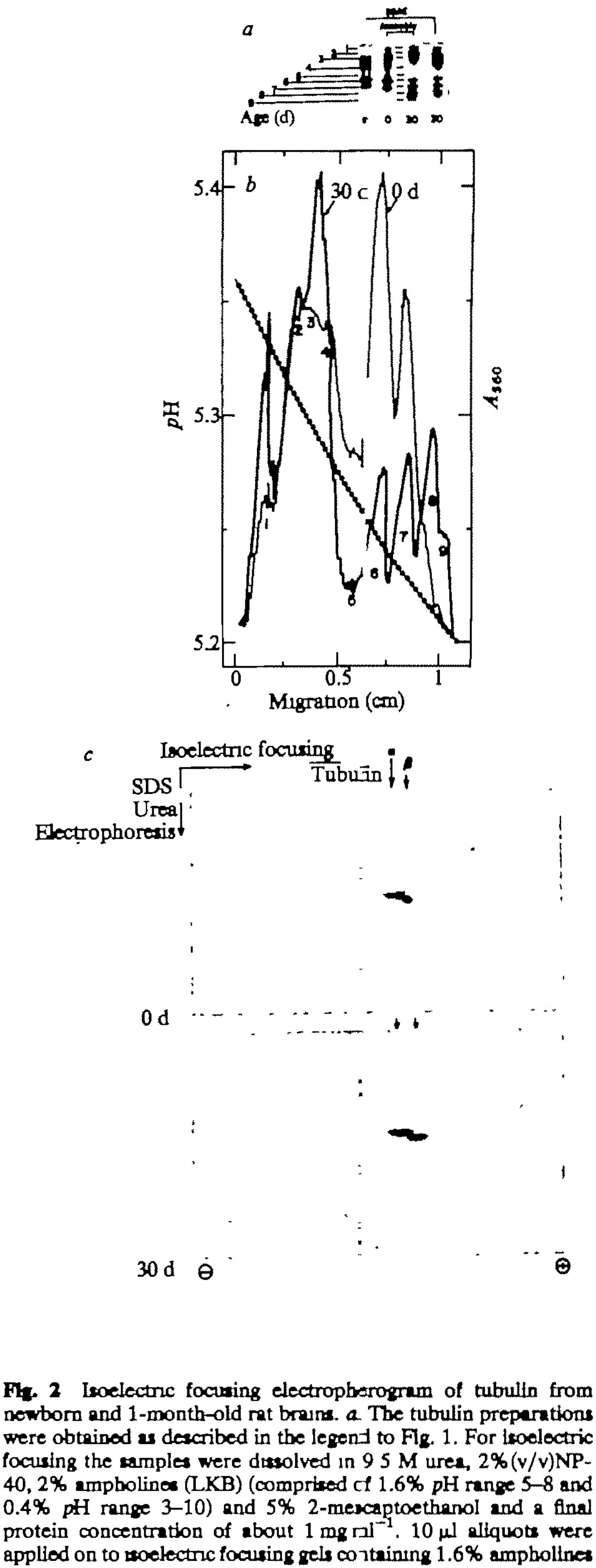

Fig. 2 Isoelectric focusing electropherogram of tubulin from newborn and 1-month-old rat brains. *a*. The tubulin preparations were obtained as described in the legend to Fig. 1. For isoelectric focusing the samples were dissolved in 9.5 M urea, 2%(v/v)NP-40, 2% ampholines (LKB) (comprised of 1.6% pH range 5–8 and 0.4% pH range 3–10) and 5% 2-mercaptoethanol and a final protein concentration of about 1 mg ml^{-1}. 10 µl aliquots were applied on to isoelectric focusing gels containing 1.6% ampholines in the pH range of 5–8 and 0.4% ampholines in the pH range of 3–10. Electrophoresis was carried out as described elsewhere[9]. On termination of the run, the gels were fixed for 60 min with 50% ethanol–7% acetic acid, soaked for 60 min with 20% methanol–7% acetic acid, stained for 5 min with 0.2% Coomassie brilliant blue–50% methanol–7% acetic acid and then destained with 20% methanol–7% acetic acid. *b*, Densitometric traces of the photographic transparencies of the isoelectric focusing gels of the DEAE-cellulose-purified tubulin were obtained by scanning at 560 nm in a Gilford 2400S spectrophotometer with a Gilford scanning attachment. The pH gradient was determined on termination of the electrophoresis (0). *c*, The DEAE-cellulose-purified tubulin separated by isoelectric focusing was subjected to 0.1% SDS–8 M urea–8.75% PAGE on a second dimension[9].

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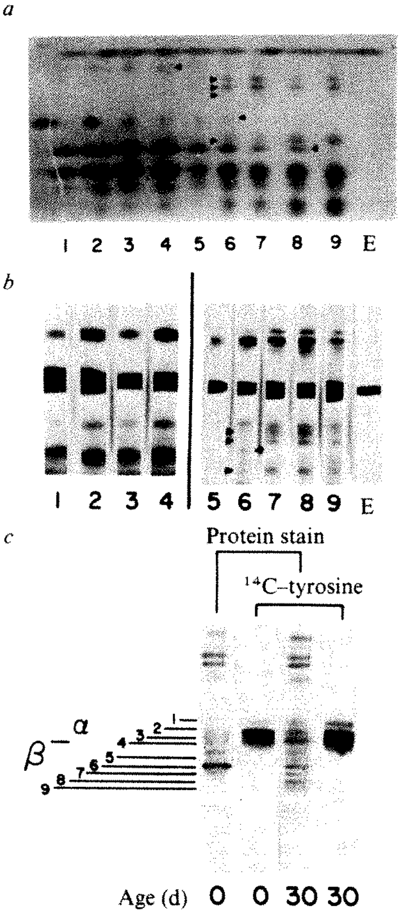

Fig. 3 Characterisation of the multiple tubulin species. *a*, *S. aureus* protease cleavage peptides of DEAE–cellulose-purified tubulin analysed on 15% polyacrylamide gel. The multiple tubulin species from 30-d-old rat brain were characterised by digestion with *S. aureus* v8 protease (36-900-1, Miles). The peptide analysis was carried out on a 15% polyacrylamide slab gel containing 0.1% SDS[19]. Tubulin bands were excised from the stained isoelectric focusing gels and soaked for 30 min in buffer containing 0.125 M Tris-HCl, *p*H 6.8, 0.1% SDS and 1 mM EDTA. The gel slices were inserted into sample slots of 15% polyacrylamide slabs with a 4-cm high upper gel. The slots were then filled with 10 μl of this buffer containing 20% glycerol and 0.001% bromphenol blue. Finally, 10 μl of the same buffer was added containing 10% glycerol and 750 ng of *S. aureus* protease. Constant voltage of 100 V was applied so that the tracking dye took 1.5 h to reach the bottom of the upper gel. The electrophoresis was then stopped for 60 min and resumed at 125 V at 10 °C and terminated 60 min after the tracking dye left the bottom of the gel. The arrow heads indicate differences in specific peptides. The isotubulins are marked in numbers according to their migration on isoelectric focusing gels. E = enzyme alone. *b*, Chymotrypsin cleavage peptides of DEAE–cellulose-purified tubulin as analysed on 15% polyacrylamide gel. The multiple tubulin species from 30-d-old rat brains were characterised by digestion with chymotrypsin (CDI-1450, Worthington). The peptide mapping was done as described above with the following modifications: 20 μg chymotrypsin were applied in each slot and the electrophoresis was stopped for 45 min instead of 60 min. The arrow heads indicate differences in specific peptides. The upper band was not marked, as its quantity varied in different experiments. The isotubulins are marked in numbers according to their migration on isoelectric focusing gels. E = enzyme alone. *c*, Tyrosylation of tubulin. Newborn and 30-d-old rat brain extracts were incubated for 10 min in the presence of L-[U-^{14}C]tyrosine (50 μCi μmol^{-1}, Radiochemical Centre), as described elsewhere[11]. The samples were diluted in 9.5 M urea, 2% (v/v) NP-40 and 2% ampholine comprised of 1.6% *p*H range 5–8 and 0.4% *p*H range 3–10 and 5% 2-mercaptoethanol. 50 μl aliquots of the suspension (final concentration ~1 mg ml^{-1}) were analysed by isoelectric focusing as described in Fig. 2, followed by autoradiography of the dried gels using X-ray film (Kodak RP-54). Note that the crude homogenate from newborn rat brain contained small amounts of band 8 which was not apparent when purified tubulin was subjected to isoelectric focusing.

by vinblastine precipitation. Purified rat brain tubulin showed only one major band of about 55,000 molecular weight on electrophoresis on SDS–polyacrylamide gels (Fig. 1). In contrast, several polypeptide chains could be resolved by isoelectric focusing. Figure 2*a*, *b* shows that tubulin obtained from newborn rat brain cytoplasm was resolved into seven bands, whereas that obtained from 30-d-old rat brain displayed nine bands. It should be noted that in both cases the resolution of bands 2, 3 and 4 was less pronounced than that of the other bands. Depending on the method of purification the relative proportion of the bands varied. Thus, bands 5 and 9 were more prominent with tubulin isolated by self-assembly, than with tubulin purified by DEAE–cellulose chromatography. However, the age-dependent increase in tubulin microheterogeneity was independent of the method of purification used. The absence of bands 8 and 9 from the young rat brain tubulin did not result from an instability factor manifesting itself during homogenisation. This was shown by purifying tubulin by DEAE–cellulose chromatography, from a 1 : 1 mixture of brain from newborn and 1-month-old rats. The purified material showed bands 8 and 9 in the proportions expected from such a mixture. Moreover, isolation of tubulin in the presence of protease inhibitors such as 0.4 mM phenylmethylsulphonyl fluoride, or a mixture of 40 μg ml^{-1} leupeptin, 20 μg ml^{-1} pepstatin A and 1 mg ml^{-1} bacitracin, did not change the pattern, indicating that the microheterogeneity does not arise from artefactual proteolysis (see also below). When the ampholine polyacrylamide gels were subjected to 0.1% SDS–8 M urea–8.75% polyacrylamide gel electrophoresis (PAGE) on a second dimension[9], bands 1, 2, 3 and 4 were found to correspond to the α-tubulin subunit and bands 5, 6, 7, 8 and 9 to the β subunit[10] (Fig. 2*c*). Thus, isoelectric focusing did not alter the tubulin mobility on electrophoresis on urea–SDS–polyacrylamide gels.

The relationship of the various tubulin bands was examined by protease digestion. After isoelectric focusing of tubulin extracted from 30-d-old rat brain the different bands were excised, subjected to *Staphylococcus aureus* protease or chymotrypsin digestion and analysed by SDS–PAGE. The results in Fig. 3*a*, *b* show a close similarity in the overall distribution of the polypeptide chains derived from each preparation, indicating a structural homology between the isotubulins and eliminating the possibility of non-tubulin contaminants. The distribution pattern of the cleavage peptides was independent of the proteolytic enzyme concentration. Moreover, comparison of the peptide maps of tubulin with non-related proteins such as actin showed marked differences (data not shown). Closer examination of the peptide maps (Fig. 3*a*, *b*) reveals differences between bands 1–5 and 6–9. In addition, band 9 differs from bands 6–8 (Fig. 3*a*) and band 6 differs from bands 7–9 (Fig. 3*b*). Although band 5 closely resembles bands 1–4 (α-tubulin), it migrates as β-tubulin when resolved by two-dimensional electrophoresis[10]. Band 5 also differs from bands 1–4 in its inability to undergo *in vitro* tyrosylation (Fig. 3*c*); this reaction is characteristic of the α-tubulin subunit[11]. All the above results suggest the existence in adult rat brain of multiple non-identical tubulin species exhibiting different isoelectric points, but similar molecular weights.

Extensive tubulin microheterogeneity seems to be prominent in the brain, as tubulin from other tissues such as 30-d-old rat liver and spleen as well as neuroblastoma–glioma NG108-15 hybrid cells were less heterogeneous and contained two major subunits (Fig. 4*a*, *b*). Peptide map analysis showed that the two tubulin species were similar to the corresponding isotubulins of rat brain, indicating no major contamination in this preparation (data not shown). The pronounced microheterogeneity of rat brain tubulin is not due to specific proteolytic cleavage occurring during the homogenisation and isolation procedures. This was shown by purifying tubulin from a mixture of ^{35}S-methionine-labelled neuroblastoma–glioma hybrid cells in the presence of 30-d-old rat brain. Figure 4*b* shows that such a mixing experiment did not produce additional radioactive isotubulins. In

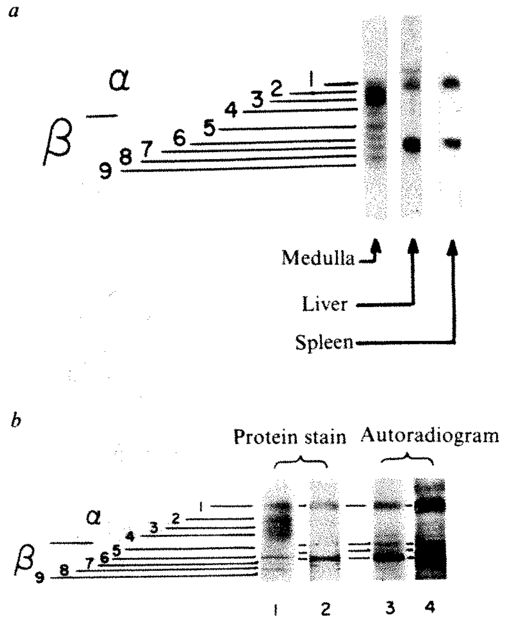

Fig. 4 Multiple forms of tubulin in various tissues. *a*, Isoelectric focusing of 30-d-old rat tubulin prepared from medulla oblongata, liver and spleen. Tubulin was purified by precipitation with vinblastine (Lilly). The various tissues were homogenised in 2 volumes of 0.25 M sucrose, 10 mM phosphate buffer, *p*H 7.4, 10 mM $MgCl_2$ and 0.2 mM GTP, and the supernatants (1 mg protein ml^{-1}) obtained after 1 h centrifugation at 105,000*g* were subjected to vinblastine (1 mg ml^{-1}) precipitation[18]. The precipitated proteins were dissolved in 9.5 M urea, 2%(v/v)NP-40, 2% ampholines (comprising 1.6% *p*H range 5–8 and 0.4% *p*H range 3–10) and 5% 2-mercaptoethanol, and a final protein concentration of ~1 mg ml^{-1}. 10 μl aliquots were applied on to isoelectric focusing gels containing 1.6% ampholines in the *p*H range of 5–8 and 0.4% ampholines in the *p*H range of 3–10. The gels were stained with Coomassie brilliant blue (as described in the legend to Fig. 2). *b*, Isoelectric focusing of tubulin from neuroblastoma–glioma hybrid cells prepared in the presence of 30-d-old rat brains. Neuroblastoma–glioma hybrid cells (NG108-15) grown as described elsewhere[20] were incubated for 24 h in the presence of ^{35}S-methionine (700 Ci $mmol^{-1}$, Radiochemical Centre), 100 μCi per 10-cm diameter plates containing 10 ml medium. The packed cells (100 μl of a pellet centrifuged at 1,000*g* for 5 min) were homogenised in 200 μl of 0.25 M sucrose, 10 mM phosphate buffer, *p*H 7.4, 10 mM $MgCl_2$ and 0.2 mM GTP, in one experiment. In a second experiment 100 μl of packed labelled cells were mixed with 0.1 g of 30-d-old rat brain and homogenised in 400 μl of the above buffer. The 105,000*g* supernatants obtained after 60 min centrifugation were subjected to vinblastine precipitation[18]. The vinblastine pellets were analysed by isoelectric focusing followed by autoradiography (as described in the legends to Figs 2, 3). 1, Tubulin prepared from a mixture of hybrid cells and brains stained with Coomassie brilliant blue; 2, tubulin prepared from hybrid cells stained with Coomassie brilliant blue; 3, autoradiogram of tubulin from hybrid cells prepared in the presence of rat brain homogenate; 4, autoradiogram of tubulin isolated from hybrid cells.

addition, the limited tubulin microheterogeneity found in neuroblastoma–glioma hybrid cells is not the result of a specific protease inhibitor. As shown in Fig. 4*b*, there was no reduction in the multiple species of brain tubulin isolated in the presence of neuroblastoma–glioma hybrid cells (Coomassie brilliant blue-stained bands).

In conclusion, an increase in the tubulin microheterogeneity was demonstrated during brain maturation. This process coincides with an increase in the ability of tubulin to undergo self-assembly[12–14], suggesting that the changes in the multiple tubulin forms control microtubule formation.

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Fibroblasts degrade newly synthesised collagen within the cell before secretion

COLLAGEN, the major extracellular protein of most tissues, is considered a relatively inert macromolecule, with a turnover rate slower than that of most proteins[1]. There is, however, a portion of the total collagen pool that is rapidly turning over, for tracer studies of collagen metabolism in intact animals have demonstrated that a significant amount of newly synthesised collagen is apparently degraded within hours of its synthesis[2]. Further, direct evidence for the rapid degradation of collagen has been provided by studies of rabbit lung explants which showed that 30–40% of newly synthesised collagen was degraded within minutes of its synthesis[3]. This phenomenon is of interest because it involves such a large proportion of collagen production, and thus may significantly influence the quantity of collagen within a tissue and hence organ structure and function. The time course of this rapid degradation of collagen suggests that it occurs within the collagen-producing cells before its secretion. Although it has been repeatedly demonstrated that intracellular degradative processes have a central role in regulating the quantity of proteins that function within cells, intracellular degradation has not been considered a general mechanism for regulating the quantity of proteins destined for extracellular function[4–6]. We describe here a direct evaluation of the hypothesis that extracellular levels of collagen are regulated, in part, by the rapid degradation of newly synthesised collagen before it reaches the extracellular space. We have studied the size and kinetics of collagen production by fibroblasts in culture, and have found that not only is a significant proportion of newly synthesised collagen degraded intracellularly, but also that the process can be modulated by manipulating the structure of the collagen molecule.

All studies used subcultivations 10–20 of a human fetal lung fibroblast termed HFL-1; this cell strain is diploid, free of

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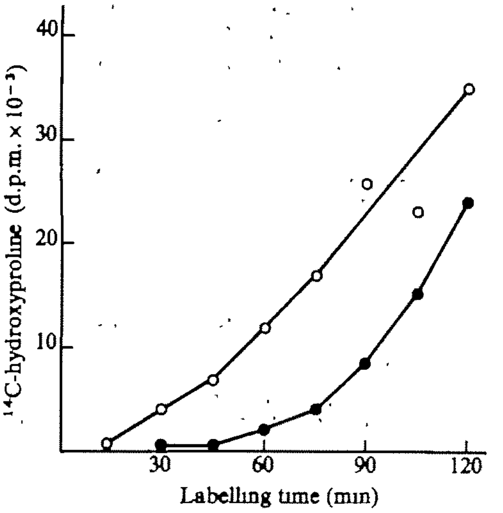

Fig. 1 Time course of the synthesis and secretion of collagen by HFL-1 fibroblasts. Collagen is represented as the quantity of ^{14}C-hydroxyproline found in the cell layer (○) or media (●) at various times following addition of ^{14}C-proline to the cultures. Cells were plated at 10^6 cells per 150-mm culture dish and maintained at 37°C (10% CO$_2$ in air) in 20 ml growth medium (Dulbecco's modified Eagle's medium (DMEM) with added fetal calf serum (10%), glutamine (0.06%), penicillin (100 U ml^{-1}) and streptomycin (100 μg ml^{-1})) which was changed every 3 d. All studies of collagen production were done after the cells had reached the stationary phase (usually 7–8 d after subcultivation) 24 h before labelling, ascorbic acid (50 μg ml^{-1}) was added to the growth medium. To evaluate collagen production by HFL-1 fibroblasts, the cells were washed (×3) with phosphate-buffered saline (PBS) *p*H 7.4, and incubated with growth medium supplemented with ascorbic acid (50 μg ml^{-1}) and *β*-aminopropionitrile (50 μg ml^{-1}). After 1 h, the supplemented growth medium was discarded and replaced with 10 ml of labelling medium (DMEM, dialysed fetal calf serum (10%), glutamine (0.06%), penicillin (100 U ml^{-1}), streptomycin (100 μg ml^{-1}), ascorbic acid (50 μg ml^{-1}), *β*-aminopropionitrile (50 μg ml)$^{-1}$ and ^{14}C-proline (10 μCi ml^{-1}, 260 mCi mmol^{-1}, Schwarz-Mann)). The dialysed fetal calf serum used in the labelling medium was prepared by dialysing fetal calf serum against PBS so as to remove amino acids that would dilute the tracer but to retain antiproteases. At each time point indicated, culture plates were removed from the incubator, placed on ice, and the culture medium collected. The cell layer was washed with PBS (×3) and the washings combined with the medium. The cell layers were scraped from the plate using 20 ml 0.5 M acetic acid. The media and cell layer samples were sonicated, heated at 100°C for 10 min to inactivate proteases, and aliquots taken for quantitation of ^{14}C-hydroxyproline using acid hydrolysis (6M HCl, 110°C, 20 h) and ion-exchange chromatography as previously described[3]. The values for ^{14}C-hydroxyproline represent the total disintegration per min (d.p.m.) of ^{14}C-hydroxyproline found in the cells or medium for each plate independent of whether the collagen was intact or degraded. The total number of cells per plate varied by less than 5%.

mycoplasma and viral infection, and exhibits contact inhibition[7]. HFL-1 synthesises both collagen types I and III, which together represent 3–5% of the total proteins synthesised by this cell. Degradation of newly synthesised collagen in HFL-1 was followed by labelling the cells, in the presence of fetal calf serum, with either ^{14}C-proline or ^{14}C-lysine and quantitating the proportion of newly synthesised proteins containing ^{14}C-hydroxyproline or ^{14}C-hydroxylysine that could pass through a dialysis membrane[3]. This experimental design for quantitating collagen degradation was based on the fact that in cultures of human fibroblasts, labelled hydroxyproline and hydroxylysine can be used as specific markers for newly synthesised collagen. Although both amino acids are found in Clq, a complement component also synthesised by the fibroblast, estimates of the relative synthetic rates for Clq and collagen demonstrate that the Clq is synthesised at 0.1% the rate (per fibroblast) of collagen[8].

As expected for a protein destined for extracellular function, newly synthesised collagen was detected in the cell layer of the HFL-1 cultures 15–30 min before its appearance in the culture medium (Fig. 1). The time course for collagen synthesis and secretion found in these cells is comparable to that of other vertebrate mesenchymal cells[9].

Strikingly, 30 min after the addition of the tracer, even though the newly synthesised collagen had not then been secreted (Fig. 1), approximately 30% of the labelled collagen in the cell layer was already degraded to peptides small enough to pass through a dialysis membrane (Fig. 2). One hour after the addition of the tracer, degraded collagen was detected in both the cell layer and the medium, but the combined total of degraded collagen represented a constant fraction of the total amount of collagen synthesised during the labelling period. Even 24 h after addition of the tracer to the cultures, the same fraction of newly synthesised collagen was found to be degraded, although by this

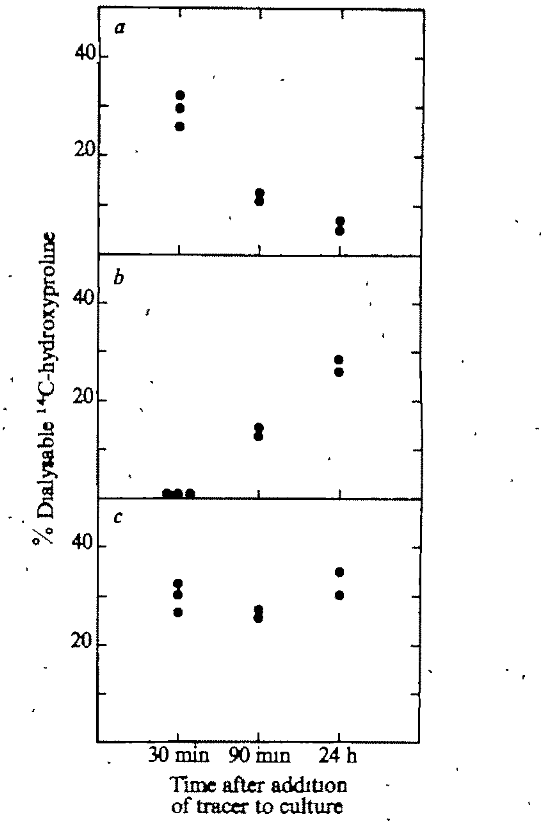

Fig. 2 Proportion of newly synthesised collagen degraded by HFL-1 cells. Cells were maintained in culture and labelled with ^{14}C-proline as described in Fig. 1. After a 30-min labelling period, the labelling medium was discarded, the cell layers were washed three times with PBS, and 20 ml of 'chase' medium (identical to labelling medium except that 1 mM unlabelled proline was substituted for ^{14}C-proline) was added. At 0, 1 or 23.5 h after addition of the chase medium (representing respectively, 30 min, 90 min or 24 h after the tracer had been originally added to the culture), the incubation was stopped and the media and cell layer collected separately as described in Fig. 1. Following sonication and heating, an aliquot was taken for analysis of ^{14}C-hydroxyproline in the *a*, cell layer, *b*, media or *c*, cell layer and media that was dialysable[3]. Per cent dialysable ^{14}C-hydroxyproline was calculated from the formula: % dialysable ^{14}C-hydroxyproline = [(d.p.m. of total ^{14}C-hydroxyproline in the sample) − (d.p.m. of ^{14}C-hydroxyproline in the sample after dialysis)] × 100/[d.p.m. of total ^{14}C-hydroxyproline in the sample]. 30 min after addition of ^{14}C-proline to the culture (that is, 9 h chase time), an average of 30% of ^{14}C-hydroxyproline in the culture was dialysable, and all was present in the cell layer. With time after the chase, the proportion of dialysable ^{14}C-hydroxyproline in the cell layer decreased and the proportion of dialysable ^{14}C-hydroxyproline in the media increased. Even so, the proportion of the total ^{14}C-hydroxyproline in the culture (cell layer and media) that was dialysable remained constant (*c*, $P > 0.1$, Student's two-tailed *t*-test).

time most of the degraded peptides had moved from the cell layer to the culture medium. Evaluation of the size distribution of the peptides derived from degraded collagen demonstrated that more than 90% of the degraded collagen was reduced to less than 500 daltons within 6 h (data not shown). Thus, not only do these cells rapidly metabolise a significant proportion of newly synthesised collagen, but they also degrade these macromolecules to very small fragments. However, a proportion of the degraded collagen was still in peptide linkage, for chromatography of the dialysable material on the amino acid analyser before acid hydrolysis resulted in a significant reduction in the ^{14}C-hydroxyproline recovered (R. Berg and R. G. C., unpublished observations). This, and the fact that the proportion of dialysable ^{14}C-hydroxyproline did not increase with time of culture (Fig. 2), are consistent with the overwhelming evidence that any hydroxyproline found in these cultures is derived from proline residues in peptide linkage (rather than an ester link such as that found in prolyl-tRNA)[10–13].

When HFL-1 cells were labelled with ^{14}C-lysine in the same conditions as with ^{14}C-proline, approximately 22% of the labelled ^{14}C-hydroxylysine was found in peptides small enough to pass through a dialysis membrane (Fig. 3). This observation is important because hydroxyproline (but not hydroxylysine) residues are present in the N-terminal ('Pn') precursor piece of collagen pro α1(I) chains (D. Hörlen and P. Fietzek, personal communication), and the finding of ^{14}C-hydroxylysine in dialysable peptides indicates that the material being degraded in these cultures includes the α-chain portion of newly synthesised collagen[3].

The fact that the degraded collagen was detected in the cell layer before the time required for HFL-1 cells to synthesise and secrete collagen argues strongly that this degradative process was occurring within, and not outside, the cells. Other evidence supports this conclusion. First, at the time degraded collagen was detected in the cell layer (Fig. 2*a*) none was found in the media (Fig. 2*b*). Although it is conceivable that collagen was degraded outside the cells and the resulting fragments then internalised, the time course of collagen degradation argues against this. Second, when purified ^{14}C-labelled human type I procollagen was incubated with HFL-1 cultures for 6 h, 100% of the labelled procollagen could be recovered intact, suggesting that the culture media did not contain active proteases capable of degrading collagen (data not shown). However, to ensure that any putative extracellular collagen-specific proteases would be inhibited, all experiments were carried out in the presence of 10% fetal calf serum, a concentration sufficient for the complete inhibition of mammalian collagenase capable of digesting 12 µg of collagen per h at 37°C (ref. 14). In addition, even in the absence of the fetal calf serum, no active collagenase could be detected in the culture media of HFL-1 fibroblasts incubated for 24 h.

Third, when the cells were pulsed with ^{14}C-proline for 30 min and then chased with unlabelled proline, the proportion of labelled collagen degraded did not change even when the cultures were continued for 24 h. If degradation was due to proteolytic enzymes outside the cells, it is likely that a progressive increase in collagen degradation would have been noted as the culture continued. The time invariance of the proportion of total collagen degraded in the pulse–chase experiments also suggested that the dialysable collagen peptides found in these cultures represented active degradation rather than simply uncompleted collagen chains 'nascent' on polyribosomes at the end of the pulse period. If it was the latter, these should have been completed during the 'chase' and the proportion of dialysable ^{14}C-hydroxyproline would have decreased.

Thus, it is apparent that a large proportion of collagen produced by diploid human fibroblasts is degraded rapidly within the cells. As this degradative process occurs before the time necessary for secretion, and as the proportion of collagen that is degraded does not change with time, it is likely that this phenomenon occurs before secretion and not after a process of secretion and phagocytosis. If the latter were responsible, the time required would be much longer and the labelled collagen in the cultures would be progressively destroyed as the culture times were extended. It is not certain, however, that all the dialysable ^{14}C-hydroxyproline and ^{14}C-hydroxylysine found in these cultures were necessarily derived from intact collagen chains. If, for example, there was premature termination of some of the collagen being synthesised, these incomplete chains might be more susceptible to degradation than fully completed pro α chains.

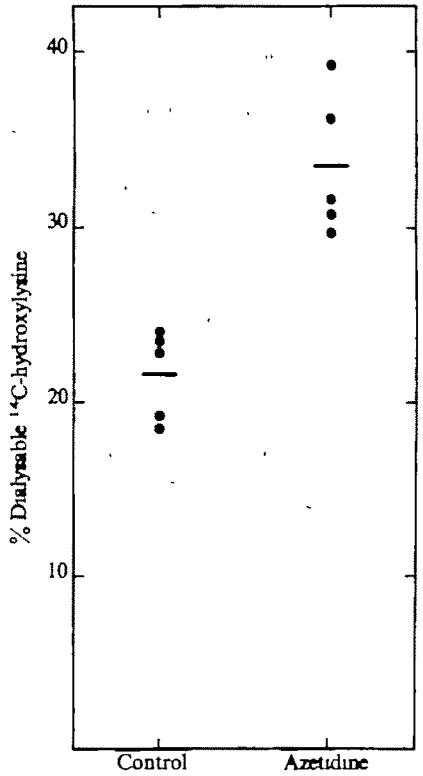

Fig. 3 Influence of azetidine-2-carboxylic acid (azetidine) on the degradation of newly synthesised collagen by HFL-1 cells. HFL-1 cells were maintained in culture as described in Fig. 1. To evaluate collagen production in the presence of azetidine, the cells were first washed three times with PBS and incubated with lysine-free DMEM supplemented with ascorbic acid (50 µg ml^{-1}) and β-aminopropionitrile (50 µg ml^{-1}). After 1 h, the supplemented growth medium was discarded and replaced with labelling medium (lysine-free DMEM, dialysed fetal calf serum (10%), glutamine (0.06%), penicillin (100 U ml^{-1}), streptomycin (100 µg ml^{-1}), ascorbic acid (50 µg ml^{-1}), β-aminoproprionitrile (50 µg ml^{-1}) and ^{14}C-lysine (10 µCi ml^{-1}, 300 mCi mmol^{-1}, Schwarz-Mann)), to which either proline (100 µg ml^{-1}, Schwarz-Mann) or azetidine (100 µg ml^{-1}, Calbiochem) had been added. Following incubation for 12 h, the cell layers and culture media were combined, sonicated and heated at 100°C for 10 min. An aliquot was taken for the measurement of total ^{14}C-hydroxylysine in the sample and the remainder dialysed against 0 5 M acetic acid and 0.1 mM hydroxylysine The proportion of newly synthesised collagen (represented by ^{14}C-hydroxylysine) that was degraded was quantitated as follows. % dialysable ^{14}C-hydroxylysine = [(d.p m. of total ^{14}C-hydroxylysine in the sample) − (d.p.m. of ^{14}C-hydroxylysine in the sample after dialysis)] × 100/[d.p.m. of total ^{14}C-hydroxylysine in the sample]. Each experimental point represents the per cent dialysable ^{14}C-hydroxylysine observed for separate culture plates The control plates (cells labelled with ^{14}C-lysine in the presence of proline) averaged 22 ± 3% and the azetidine plates (cells labelled with ^{14}C-lysine in the presence of azetidine instead of proline) averaged 33 ± 5%, the values were significantly different at the $P < 0.01$ level (Student's two-tailed t-test).

If intracellular collagen degradation is an important post-translational mechanism which modulates the differentiated state of fibroblasts with respect to collagen production, these cells should have means available to vary the relative proportion of newly synthesised collagen that is degraded by this process.

There is accumulating evidence that intracellular degradative processes can recognise and destroy protein with abnormal conformations[4], so that one way to evaluate whether cells can alter intracellular collagen degradation is to manipulate the cells to produce collagen molecules with an abnormal structure. The approach used was to incubate HFL-1 cells in medium in which azetidine-2-carboxylic acid had been substituted for proline. Azetidine, the lower homologue of proline, is readily taken up by cells, recognised by prolyl-tRNA, and incorporated into newly synthesised collagen in place of proline[15]. However, the presence of the azetidine ring in the collagen polypeptide chain precludes normal helix formation[16,17]. Interestingly, when this was done, intracellular collagen degradation increased almost twofold (Fig. 3). Thus, cells seem to have means by which they can modulate the fraction of total collagen that is degraded within the cell before secretion.

As might be expected for a macromolecule with such a critical role in organ structure and function, there seem to be several ways in which the metabolism of collagen can be regulated. For collagen already outside the cell, extracellular proteases such as collagenase are undoubtedly paramount in this regard[18]. The present study suggests that mechanisms are also available by which cells can degrade a significant, yet adjustable, proportion of newly synthesised collagen before it ever reaches the extracellular space. Further study will be required to ascertain whether this is a general control phenomenon for secreted proteins or whether it is unique to collagen.

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Nitrogen fixation in the rhizosphere of rice

THE discovery of nitrogen-fixing activity associated with the rhizosphere of non-leguminous plants such as tropical grasses, rice and maize has provoked considerable interest[1–9]. Discrepancies in the measurements of this nitrogen-fixing activity by the acetylene reduction assay in different conditions prompted us to develop a modified assay system. We report here the use of this assay to find significant differences in the rates of acetylene reduction associated with the rhizosphere of 50 different strains of rice. This suggests that the rice plant genotype influences the association with nitrogen-fixing bacteria in its rhizosphere.

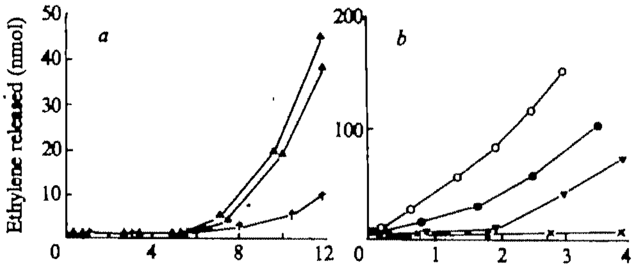

Fig. 1. Time course of C_2H_2-reducing activity in different assay conditions. C_2H_2-reducing activity was measured by gas chromatography (Shimadzu GC-3BF) with H_2-flame ionisation detector. Assay conditions were a 2-cm column containing Porapack Q of 80–100 mesh with N_2 gas as carrier at a flow-rate of 40 cm³ min^{-1} at 80 °C. *a*, The gas phase was replaced with a mixture of 0.1 atm C_2H_2 and 0.9 atm He and incubated at 30 °C until assayed. (▲), Excised roots washed with tap water and kept in a flask, per g dry root; (△), whole plant with soil kept in a polyethylene bag, per g dry root; (+), soil alone kept in a flask, per 100 g. *b*, Whole plant kept in a vessel containing different gas phase (per g dry root). Gas phase: 0.1 C_2H_2 and 0.9 He (○); 0.1 C_2H_2 and 0.9 air (●); and after the gas phase was replaced with 1 atm air and kept for 30 min in order to expose rhizosphere to air, it was replaced again with a mixture of 0.1 C_2H_2 and 0.9 He (▼). Control, no C_2H_2 (×).

The first report of high levels of nitrogen-fixing activity associated with isolated roots of maize was by von Bülow and Döbereiner[4]. Subsequently, using the maize–*Spirillum lipoferum* system, Burris *et al.*[10] showed that the acetylene-reducing activity of intact plants or soil cores containing the bulk of a relatively undisturbed root system was far less than that of preincubated roots. Similar observations were made in rice. When the excised root system or intact plant kept in a polyethylene bag was preincubated under either aerobic or anaerobic conditions, the activity remained almost zero for the first few hours after incubation[8,11–13] and increased exponentially on further incubation. In the maize–*Spirillum* system, a marked proliferation of the bacteria during preincubation was observed[14,15]. This suggested that the activity detected after a lag period might be due to the increased number of bacteria and might not represent the activity in the intact plant.

We devised the following assay system with these observations in mind. An intact plant including the soil and water of the rice rhizosphere and the bulk of the relatively undisturbed root system was enclosed in a vessel such as a desiccator so that the exposure of the rhizosphere to air was minimised. Rigorous displacement of the gas phase in the vessel with a mixture of helium and acetylene (9:1 by volume) was achieved two times by an evacuation pump (up to 0.02 atm) and flushing the gas mixture up to 1 atm. The vessel was flushed with the gas mixture for 30 min to equilibrate the system, evacuated and flushed again. The system was incubated in normal laboratory light at 28 °C and fractions of the gas phase were withdrawn with a syringe for assay. The ethylene produced from acetylene was measured by gas chromatography[16,17]. It was thought that the assay procedure enabled us to displace the air remaining in the assay sample with the gas mixture containing acetylene as a substrate so that the substrate could effectively reach the inner part of the sample where nitrogen fixation was active without disturbing the rhizosphere. The observations described below suggest that the above two assay conditions must be fulfilled to measure the nitrogen-fixing activity in rhizosphere correctly.

Figure 1 shows the kinetics of acetylene reduction in the rice rhizosphere measured in the various assay conditions. When the assay system described above was used, considerable acetylene reduction activity was found with certain strains of rice. The production of ethylene from acetylene was linear and no time lag in the reaction was observed. The activity detected by this method was found in conditions in which almost no time was allowed for bacterial growth in the system. On the other hand,

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the other assay system using polyethylene bags[13] or excised roots in a flask[4,12] showed a distinct time lag before ethylene production was detected. When acetylene was not added to the system, no ethylene was produced. Soil without plants showed no detectable acetylene reducing activity during a 6-h incubation and later small but significant amounts of ethylene were observed. Aerobic conditions for acetylene reduction gave lower activity than anaerobic conditions. Exposure of the rhizosphere to the air for 30 min also reduced activity considerably. Plants were incubated in the light and it is possible that photo-produced oxygen is altering the physiological environment of the rhizosphere to favour microaerophilic bacteria. This, however, is unlikely as our whole plant assay system showed linear acetylene reduction without a lag. From this we deduce that our assay system measures functional nitrogenase activity in the rhizosphere and provides a useful method to assay the nitrogen-fixing activity of the plant–rhizosphere complex.

The nitrogen-fixing activity of the rhizosphere of 50 strains of rice was then measured. The strains used were collected from various parts of tropical Asia and preserved at the Genetic Stocks Centre, National Institute of Genetics, Mishima. The plants were grown in pots until they flowered and the activity of these intact plants, two of each strain, was then measured in our assay conditions. Measurements were taken 1 and 2 h after incubation and the amount of ethylene produced per hour per plant was calculated. The values obtained early after incubation reflected the activity of the rhizosphere as then any activity caused by the growth of nitrogen-fixing bacteria could be ruled out. The rate of nitrogen fixation varied widely between strains (Table 1). In addition, 20 pots containing soil without plants were measured as controls. No activity was detected in these pots during the first few hours of incubation at room temperature. Results shown in Table 1 were measured within 2 h of incubation. There was, therefore, a distinct difference in the nitrogen-fixing activities between controls and experimental pots. The variation among strains was statistically significant (Table 2). Activity expressed per g dry root per hour also showed a wide range of significant variation between strains. The highest value was about 1,100 nmol per plant per hour. Strains showing high activity were collected in India and Thailand. However, the value was rather low compared with those (2,000–6,000 nmol per g dry root per h) obtained by Dommergues *et al.*[9] using rice strain IR 8. Also using IR 8, Lee *et al.*[13] reported lower values (1,870 nmol per pot per h). Because the soil used in those experiments and experiments reported here might have contained different microbial floras and the activity was also measured in different assay conditions, it is difficult to compare these results.

There was a correlation ($r = 0.895$, significant at the 1% level) between root weight and nitrogen-fixing activity within a strain. This means the larger the amount of root per unit area, the higher the activity. Roots are, therefore, apparently essential for bacterial nitrogen fixation in the paddy field.

Seeds of a rice strain associated with high nitrogen-fixing activity were sterilised and grown in sterilised soil under germ-free conditions. Nitrogen fixation was measured. No acetylene reduction was detectable on the sterile plant, suggesting that microorganisms must be involved. Although no nodules were found on the roots of rice associated with high nitrogen-fixing activity, an anaerobic, rod-shaped, Gram-positive, biotin-requiring bacterium, which formed terminal spores was isolated. This is thought to be a *Clostridium*. It is known, however, that from a single soil sample one can isolate many species of bacterium capable of fixing nitrogen, such as *Desulfovibrio*, *Mycobacterium* and *Beijerinckia*, and it is not yet possible to detect which bacterium is responsible for nitrogen fixation in the rice rhizosphere. A systematic examination of nitrogen-fixing bacteria in the rhizosphere of rice in our experimental field is in progress.

Table 1 Variation in acetylene reducing activity of 50 rice strains

C_2H_2 produced (nmol per plant per h)	No. of strains
0–200	13
200–400	23
400–600	8
600–800	2
800–1,000	3
1,000–1,200	1

Acetylene reducing activity of strains was obtained by averaging the measurements of two plants obtained during the flowering period.

Table 2 Analysis of variance of acetylene reducing activity among 50 rice strains

Source of variation	d.f.	Sum of squares ($\times 10^{-3}$)	Mean square ($\times 10^{-3}$)
Total	99	8,152	
Strain	49	5,576	113.8*
Error	50	2,576	51.5

* Statistically significant at the 1% level.

The significant variation found among rice strains in their degree of association with nitrogen fixation could suggest that the genotype of the plant influences this association. A readily available source of energy is crucial in establishing the association of nitrogen-fixing microbes with rice. If the rice plant excretes available substrates through its root system, microorganisms would take advantage of this to grow and fix nitrogen in conditions which do not repress nitrogenase synthesis. Certain types of plant excrete sufficient carbohydrate to support a sizeable microbial population[3,18]. Not only *Azotobacter* and *Spirillum* benefit, but also strictly anaerobic organisms such as *Clostridium* and facultative organisms[19,20]. The significant correlation between the activity of nitrogen fixation and root weight as well as the genotype of the rice plant could be due to a dependence of nitrogen-fixing microbes on the energy source provided by the plant. As a great deal of energy is needed to support microbial growth and nitrogen fixation, the energy source will be the limiting factor for the plant–bacterium association.

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Identification of a new chicken α-globin structural gene by complementary DNA cloning

THE identification of a new chicken α-globin gene is reported here. Nucleotide sequence analysis of clone pHb1003 shows it to code for an α-subunit with an amino acid sequence different from those normally observed in the adult (the αA and αD). Characterisation of a second clone, pHb1010, carrying the same novel sequence, suggests that this mRNA sequence was present in substantial quantity after very young (2–3-week-old) chickens were treated with phenylhydrazine. The results are therefore similar to those reported previously for sheep and goats, where treatment with phenylhydrazine results in the turn-on of a new stress globin gene. As the ease of obtaining nucleated erythrocytes from avian systems facilitates many types of biochemical studies, we believe that this finding may provide an especially useful system in which to study the molecular events involved in globin gene switching.

Our laboratory has previously reported the complementary DNA (cDNA) cloning of globin mRNA sequences from phenylhydrazine-treated 2–3-week-old white Leghorn chickens[1]. One of these clones, pHb1003, was shown by the ladder DNA sequencing technique of Maxam and Gilbert[2] to contain an α-globin cDNA insert. This determination was based on the discovery of a nucleotide sequence within a pHb1003 *Hae*III insert band which codes for amino acid residues 86–98 of the chicken αA primary structure as determined by Matsuda[3]. These amino acids are highly conserved in most vertebrate α-globin proteins[4]. As such, this sequence is useful for identifying pHb1003 as an α-globin insert, but it does not indicate whether this DNA is derived from the αA or another α gene. Other positions along the α-globin molecule are more variable, most notably positions 102–125, and 46–82 (ref. 4). Restriction mapping data (Fig. 1) enabled us to locate and sequence pHb1003 DNA fragments from the latter variable region. Using the ladder technique, sequences were obtained from a 510-base pair *Alu*I fragment coding for the untranslated region at the 5′ end of the mRNA and amino acids 1–83 of the α globin (Fig. 2). The 69-base pair sequence obtained codes for amino acid residues 60–83 of the α globin. It includes 10 positions where the amino acid sequence is not compatible with the αA sequence and 16 positions which diverge from the αD sequence[5] (Fig. 3). The presence of so many differences argues against the idea that we are observing an allelic form of either the αA or αD genes.

As the αA and αD subunits account for 70% and 30%, respectively, of the α-subunits normally present in adult chicken reticulocytes[6,7], this raises several interesting possibilities. The polypeptide coded by pHb1003 might represent a minor normal

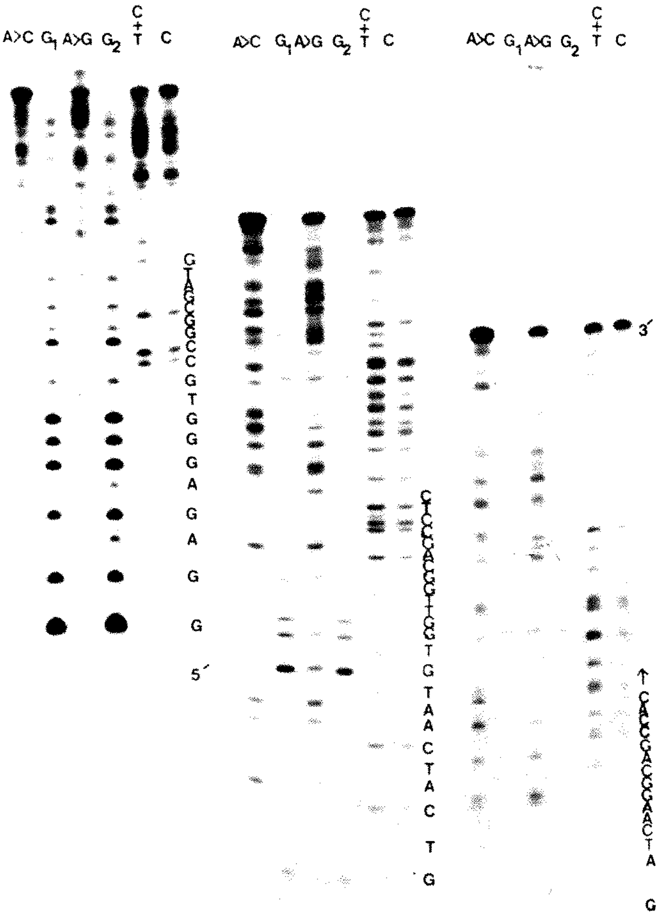

Fig. 2 Autoradiogram of the ladder sequence from a 480-base pair *Alu*I pHb1003 insert band. 100 μg of pHb1003 was digested with *Alu*I and the products were fractionated on a 6% acrylamide 1/28 bisacrylamide preparative gel. The 510-base pair insert fragment was eluted from the gel in high salt overnight. The 5′-terminal phosphates were removed by treatment with bacterial alkaline phosphatase and replaced with the ^{32}P label of λ ^{32}P-ATP using T4 polynucleotide kinase. This DNA, which was labelled at both 5′ termini, was cleaved with *Hae*III, giving two single-end-labelled fragments of 320 and 190 base pairs. The 190-base pair DNA was aliquoted into 5 equal fractions. The aliquots were separately subjected to partial chemical cleavages specific for G, A, A>C, C+T and C. The partial cleavage products were then loaded side by side on 20% acrylamide 1/60 bisacrylamide 7 M urea gels at 3 successive intervals. The radioactive bands were visualised by autoradiography at −70 °C with Kodak Hi-Plus tungstate intensification screens. The nucleotide sequence is read from the 5′ end at the bottom of the gel to the 3′ end at the top. The sequence shown is complementary to the pHb1003 coding strand.

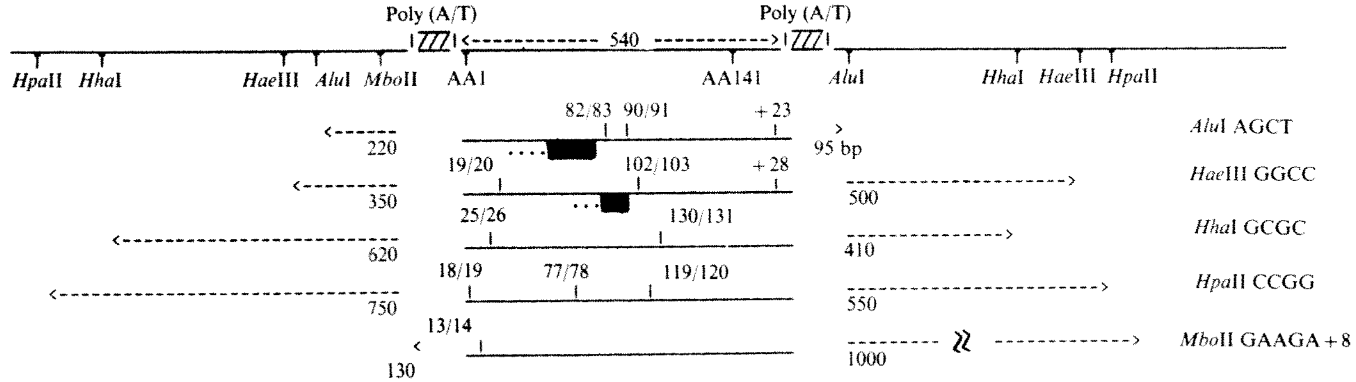

Fig. 1 Restriction map of the pHb1003 α-globin cDNA insert. Insert fragments were identified and sized by electrophoresis of various pHb1003 restriction digests alongside similar cleavages of the pMB9 vector on 6% acrylamide 1/29 bisacrylamide gels. Secondary digests of purified insert cleavages occurring within the 3′ untranslated region are denoted by a '+' followed by the site's distance from the UAA termination codon (in base pairs). Heavy lines are used to indicate the regions which have been sequenced. The exact locations of those restriction sites which have not been sequenced were estimated by extrapolating from those in regions where the nucleotide sequence is known and fitting with the amino acid sequence. The numbers indicate base pairs.

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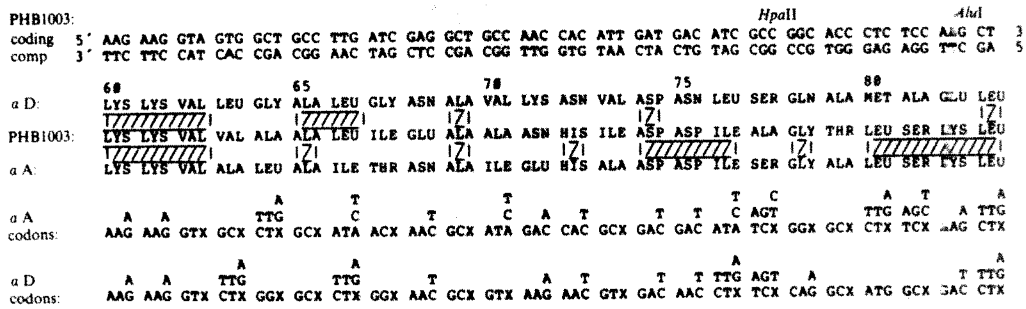
PHB1003:
HpaII
AluI
coding 5′ AAG AAG GTA GTG GCT GCC TTG ATC GAG GCT GCC AAC CAC ATT GAT GAC ATC GCC GGC ACC CTC TCC AAG CT 3′
comp 3′ TTC TTC CAT CAC CGA CGG AAC TAG CTC CGA CGG TTG GTG TAA CTA CTG TAG CGG CCG TGG GAG AGG TTC GA 5′
60 65 70 75 80
α D: LYS LYS VAL LEU GLY ALA LEU GLY ASN ALA VAL LYS ASN VAL ASP ASN LEU SER GLN ALA MET ALA GLU LEU
PHB1003: LYS LYS VAL VAL ALA ALA LEU ILE GLU ALA ALA ASN HIS ILE ASP ASP ILE ALA GLY THR LEU SER LYS LEU
α A: LYS LYS VAL ALA LEU ALA ILE THR ASN ALA ILE GLU HIS ALA ASP ASP ILE SER GLY ALA LEU SER LYS LEU
A T T T C A T A
α A A A TTG C T C A T T T C AGT TTG AGC A TTG
codons: AAG AAG GTX GCX CTX GCX ATA ACX AAC GCX ATA GAC CAC GCX GAC GAC ATA TCX GGX GCX CTX TCX AAG CTX
A A A A
α D A A TTG TTG T A T T T TTG AGT A T TTG
codons: AAG AAG GTX CTX GGX GCX CTX GGX AAC GCX GTX AAG AAC GTX GAC AAC CTX TCX CAG GCX ATG GCX GAC CTX

Fig. 3 Nucleotide sequence from the 5′ terminus of the 510-base pair *Alu*I pHb1003 insert fragment shown in Fig. 1. The corresponding polypeptide sequence is shown juxtaposed to the αA and αB globin primary sequences reported by Matsuda and Takei[3,5]. Sequence homology is indicated by the cross-hatched bars. The amino acids listed as evolutionarily conserved by Dayhoff[4] are enclosed in rectangles. The possible codons for amino acids 60–83 of the αA and αD are shown below the amino acid comparisons.

adult globin chain or one not previously recognised in 2–3-week-old chicks. Alternatively, it is possible that the stress of phenylhydrazine treatment causes these young chickens to synthesise other globin chains, as is known to be the case in sheep or goats. If so, this stress globin might represent activation of a globin gene not previously identified, or it might represent renewed synthesis of embryonic π or π' α-globin subunits or of haemoglobin H (the α subunit of which is uncharacterised).

The observed variations cannot easily be explained as the result of rearrangements in the cloned DNA; it is unlikely that such rearrangements could cause the observed variations while preserving the correct reading frame, the conserved residues at positions 69, 74, 80 and 83, and the proper spacing between these conserved residues. Nor is it likely that these variations are due to polypeptide sequencing errors: the amino acid sequence in question is bracketed by a tryptic fragment from Lys 61 to Lys 81 and a chymotryptic fragment from Leu 65 to Leu 80. Both these fragments are small and easily sequenced by the Edman (phenylisothiocyanate) and Sanger (dinitrofluorobenzene) methods used by Matsuda *et al.*

To test the hypothesis that pHb1003 might represent only a minor globin sequence, we have carried out a preliminary characterisation of other chicken globin cDNA clones. Twelve globin-insert-carrying plasmids identified from colony hybridisation studies were grown and analysed by cleavage with the restriction enzyme *Hae*III. Of the restriction patterns obtained, one showed a 248-base pair fragment characteristic of pHb1003. All but one of the remaining plasmids contained β-globin inserts as shown by the presence of a 66-base pair *Hae*III fragment. The remaining plasmid, pHb1008, showed a unique pattern and may represent an entirely different α- or β-globin gene sequence (A.Y.L. and Lee, unpublished results). The discovery of a second plasmid, pHb1010, with a restriction pattern similar to that of pHb1003 indicated that this is not a minor sequence in this mRNA preparation. It also made it possible to test directly the possibility of rearrangements in the cloned DNA. The ladder sequencing technique was used to compare the nucleotide sequences of plasmids pHb1003 and pHb1010 in the same region shown in Fig. 3. The sequence of pHb1010 was found to be identical to that found for pHb1003 in this region, but different from the sequence expected for the αA or αD mRNAs, as discussed earlier.

The fact that nine clones out of 12 showed the β-globin restriction pattern suggests two possibilities. The first is that the α-globin sequences were cloned at low frequencies, in which case we might have lost a specific class of α-globin mRNA. The alternative possibility is that α and β sequences were cloned with similar efficiencies but that the α clones give slightly lower signals in the colony hybridisation test so that they are somewhat under-represented among the 13 clones chosen for the most intense signals. Our experimental results show that this latter possibility is correct. When the colony hybridisation test was repeated with probes specific for the α- and β-globin genes (restriction fragments from pHb1001 and pHb1003 were used as probes), approximately equal numbers of α- and β-globin clones were observed (I.W.C., unpublished results).

On the basis of these results, we favour the hypothesis that the stress of phenylhydrazine treatment in these young chickens may cause the turn-on of synthesis of a stress globin. The complete sequence determination of the pHb1003 clone, which is now underway, will be helpful in establishing whether it codes for the embryonic π or π' α-globin subunits, or the α subunit of haemoglobin H, or for a novel stress globin such as occurs in sheep or goats. Interestingly, the amino acid variations observed between positions 60 and 83 generally retain the polarity and charge observed in the corresponding region of the αA polypeptide sequenced by Matsuda *et al.* With the exception of a Thr-to-Ile shift at amino acid residue 67, all the replacements combine to prevent an overall change in polarity or charge. The Asn-to-Glu change at residue 68 is opposed by a Glu-to-Asn change at position 71. The Ser-to-Ala shift at amino acid 77 is compensated by an Ala-to-Thr change at position 79. The remaining changes at residues 63 64, 70 and 73 all involve uncharged nonpolar amino acids.

Amino acids 60–83 occur in the EF 'corner' region of the α-globin polypeptide between the E and F helices (residues 52–71 and 80–88, respectively, by Kendrew's nomenclature[9,10]). The EF region bends around the haem and forms a part of the hydrophobic centre of the intact haemoglobin molecule. This suggests that for the chicken α-globin polypeptide the maintenance of correct charge and polarity in such bend regions may be more important than other features of protein stereochemistry.

This work was supported by USPHS grants GM 18586 and HL 21831. A.L. was supported by NCI predoctoral training grant 4–783825–32923–7.

Note added in proof: One of us (A.Y.L.) has completed the DNA sequence of pHb1003 (manuscript in preparation). The polypeptide sequence encoded by this plasmid insert differs from the adult αA globin at 23 positions and from the αD at 63 positions. At the same time the predicted tryptic cleavage products of this sequence would differ from those reported for the π and π' embryonic α-type globins. We are therefore led to conclude that pHb1003 encodes a previously uncharacterised αA-like globin.

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matters arising

Seismic evidence for Mesozoic sedimentary troughs on the Hebridean continental margin

JONES'S interpretation of seismic refraction lines on the NW British continental shelf[1] provides welcome corroboration of the Mesozoic sedimentary basin between the Flannan Isles and Lewis first postulated by Bullerwell (see refs 2–4). As we have already suggested that the Rockall Trough developed by seafloor spreading in the early Permian[5–7], we wish we could also welcome Jones' interpretation of the structure of the NW British margin, which suggests that the development of the Trough, at least in part, goes back to Permo-Triassic times. However, we interpret the high velocity refractor ($V_P = 4.4 \pm 0.3$ km s^{-1}) as corresponding merely to the top of a layer of Palaeocene–Eocene basalts, because (1) regional mapping and study of interval velocities using commercial 24-fold reflection lines and aeromagnetic coverage shows that basalts extend southwards from the Faeroes over almost the whole of the Rockall Trough north of Anton Dohrn seamount (as was suggested by Roberts[8]) and also over the outer NW Scottish shelf from the Wyville–Thomson Ridge to about 58° N; (2) the high-frequency negative magnetic anomaly field characteristic of these basalts at shallow depth is clearly seen both on Jones' magnetic profile[1] and on the IGS aeromagnetic map[2]; and (3) the refractor velocity is similar to that for basalts elsewhere on the NW European margin[9–11], but probably too high for Permo-Triassic sediments; although the interval velocity for pure Permo-Triassic sandstones at ~4 km depth in wells in the Viking Graben[12] and West Shetland Basin[13] can exceptionally attain values as high as 4.5 km s^{-1}, the usual range of velocity is 3.6 – 4.1 km s^{-1} for Permo-Triassic sandstones in these areas. The Permo-Triassic beneath the Little Minch has a $V_P \sim 3.9$ km s^{-1}, obtained by refraction shooting[14]. Above the basalts, the refractors with apparent $V_P \sim 2.8$–3.1 km s^{-1} could be due to Quaternary glacial till; measurements on data from the West Shetland area show that glacial till up to 100 m thick can have interval velocities $V_{\text{int}} \sim 2.6$–2.9 km s^{-1}. However, the Tertiary sediments between the Quaternary and the basalts have a lower V_{int}, so the top of the basalts is somewhat shallower than that given by Jones' profiles.

The gravity suggests that there may be sediments beneath the basalts, but we have no way of estimating their age. Thus we conclude that Jones' results have no relevance to the early evolution of the Rockall Trough, and, furthermore, his attempt to estimate the hydrocarbon prospects of this part of the margin is misleading; prospects here are poorer than elsewhere, because of the basalt cover. However, our reconstruction[7] suggests that in mid-Jurassic times Orphan Knoll was situated at the south-east margin of the Rockall Trough. The neritic Bajocian sands found beneath the Knoll[15] may therefore occur elsewhere along the margins of the Trough, and may also overlie downfaulted basins of Carboniferous age on the outer continental margin, developed during an intracontinental rifting phase[6] before early Permian spreading. Therefore along the margins clear of Tertiary basalt cover, for example, the south-east Faeroe–Shetland Trough margin between 60 and 62° N, and both margins of the Rockall Trough south of about 58° N, the hydrocarbon prospects may be very good.

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JONES REPLIES—The uncertainties involved in estimating stratigraphical ages from seismic velocities in the Hebridean region were emphasised in my paper[1] and I am therefore grateful to Smythe *et al.* for their early comments. Although these are primarily based on their unpublished reflection profiles, velocity data and regional maps, some further pertinent remarks can be made.

Smythe *et al.* accept my conclusion that Mesozoic sediments occur immediately west of Lewis, an area in which the age of the sediment cover was previously undefined[2]. My inference that Mesozoic sediments also lie close to the sea floor north-west of the Flannan Ridge is based on the observation that the 2.8–3.1 km s^{-1} values on lines JM-9 and JM-10B are significantly higher than published velocities in Tertiary–Quaternary sequences[1]. Thus constrained, I was unable to interpret the deeper 4.0–4.4 km s^{-1} refractor as the top of a pile of Tertiary basaltic lavas. Using 'measurements on data' from an unspecified location near the Shetlands, Smythe *et al.* attribute the 2.8–3.1 km s^{-1} values to glacial till, which then allows them to suggest that the 4.0–4.4 km s^{-1} layer is much younger than I proposed. As they do not indicate the water depths in which the high Pleistocene velocities were determined, the method of measurement and also local velocity changes, it is not clear whether a direct comparison with shallow refractor velocities near the Flannan Isles is justified. Are their 2.6–2.9 km s^{-1} values sufficiently typical of the Pleistocene to permit an extrapolation over a large area? Their results may be of regional importance but as careful mapping by Eden[3] and others has revealed marked and often rapid lateral variations in Pleistocene deposits off northern Britain, Smythe *et al.* need to provide additional evidence to show that thick, high velocity boulder clay is likely to lie near the shelf break, some 300 km south-west of the Shetlands. They must also account in their interpretation for the presence of pre-Quaternary reflectors[4,5] within the westwards continuation of their proposed Quaternary sequence.

In their discussion of the 4.0–4.4 km s^{-1} refractor, four references are misleadingly quoted (their refs 10–13) to add weight to their contention that it represents Tertiary basaltic lavas and not pre-Jurassic sediments. Seismic velocities for basalts are omitted in ref. 10. Talwani and Eldholm[11] ascribe velocities near 4.4 km s^{-1} on the Norwegian margin, not to basalts, but to pre-Cretaceous sediments. A limiting 4.5 km s^{-1} for Permo-Triassic sediments is not included in their refs 12, 13. My values of 4.0–4.4 km s^{-1} seem to be well

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within the permissible range for Permo-Trias as the refraction profiles of Browitt[6] show velocities of 4.6–4.8 km s^{-1} at a level in the West Shetland Basin where Cashion's sections[7] reveal thick sediments of this age. Thus, pre-Jurassic deposits cannot be dismissed at the depths I have indicated. They correctly note that magnetic anomalies occur over the 4.0–4.4 km s^{-1} layer but, rather than arising from Tertiary lavas, these can be interpreted as reflecting small basic intrusions within a thick Mesozoic section and magnetisation contrasts in the Precambrian basement.

In denying that my results are relevant to the early evolution of the Rockall Trough, Smythe *et al.* have ignored my demonstration of major faulting and crustal subsidence. As they accept a Mesozoic age for the Flannan Trough they must also accept the existence on the continental margin of NNE-trending faults which were active during Mesozoic time. As these run approximately parallel to the continental slope north of St Kilda and have increasingly larger displacements northwestwards[1], it is difficult to resist the conclusion that the faulting is directly associated with the major phase of downwarping in the Rockall Trough. My section showing thick pre-Jurassic sediments north-west of the fault-bounded Flannan Ridge is compatible with the Permian opening first suggested by Heirtzler and Hayes[8] but it does not establish that seafloor spreading took place at this time.

Commenting on petroleum prospects off northern Britain, Smythe *et al.* offer a comparison with other parts of the Rockall Trough that hinges on unpublished data and a geometrical argument which is questionable because the extent of oceanic crust in the region is unknown. Several parts of the Rockall Trough clearly deserve detailed commercial exploration, although my appraisal of prospects would be less sweeping than that given by Smythe *et al.* Moreover, in view of the geophysical indications of thick sediments and major Mesozoic faulting west of Hebrides, it seems premature for them to take a firm, pessimistic stand on a large section of the Scottish margin, especially since drilling investigations are at such an early stage.

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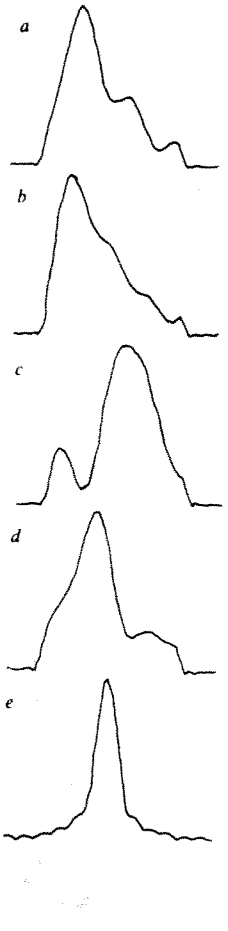
Object reconstruction from intensity data

GULL AND DANIELL[1] have recently proposed a new maximum entropy algorithm for the reconstruction of an object field from incomplete and noisy measurements of its Fourier transform. This new algorithm may encourage other workers to apply maximum entropy techniques to their own problems. For this reason we point out here that the application of maximum entropy techniques for object reconstruction in the absence of phase information could lead to wholly false conclusions due to ambiguities. We shall illustrate some of the problems using a specific example. Many such examples may easily be generated and the one chosen here is not unusual.

Figure 1 shows four different, real and positive objects which all give rise to the scattered intensity shown in Fig. 1*e*. Families of dissimilar objects whose Fourier transforms have the same modulus occur whenever the scattered intensity has non-zero minima[2]. Such minima indicate the presence of complex zeros in the scattered field. For each such zero there are two possible positions, which correspond to different objects whose Fourier transforms have identical moduli. The possible positions for these zeros are symmetrical with respect to the real axis and thus one speaks of 'flipping' zeros about this axis in order to generate different objects. The example in Fig. 1 was produced by 'flipping' such zeros. Clearly the four objects thus created are very different, for example, in the number and position of their peaks.

Fig. 1 All four different real positive objects shown in *a*, *b*, *c* and *d* produce the same far field intensity, shown in *e*. The four different objects have been generated by reflecting or 'flipping' the zeros of $F(z)$ about the x-axis, as described in the text.



The maximum entropy algorithm should converge to one of these possible solutions but the only criterion it has as a basis for the selection of that solution is that of smoothness, as all the possibilities are real and positive. However, inspection of the four possible solutions shows that none may be disregarded as unphysical—they are all 'smooth'. It would only be possible to identify one of these possibilities as the solution if one has some extra information, such as approximate phase values which may provide a basis for such discrimination. On the basis of the intensity alone there can be no unique solution, unless all minima are zero. The selection of one solution using an entropy criterion is dangerous. For example, if the algorithm chose the third solution one might conclude that the object consisted of two distinct peaks, whereas the other possible solutions show that such a conclusion is unfounded.

The example given here is simpler than may be expected in general—there are few complex zeros—and thus 'real' data may be expected to result in even greater ambiguity. In these conditions it is vital that some measure of the ambiguity is established. The maximum entropy algorithm does not give such a measure[3].

For convenience the analysis used to produce the example in Fig. 1 is one-dimensional but similar ambiguities will exist in two-dimensional systems.

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Human reproduction reconsidered

MAY'S article in News and Views, '*Human Reproduction Reconsidered*'[1], fails to mention that the research on the demography of the !Kung hunter-gatherers, reviewed by Short and by

Kolata, is from my work. A brief report can be found in *Kalahari Hunter-gatherers*[2]. A full account, including data, methods and analyses (including a comparison with the Hutterites and a consideration of the implications of the Frisch hypothesis) will be found in my forthcoming book[3].

The figure of 15.5 yr for the mean age at menarche for !Kung girls (quoted by Kolata from a telephone interview) has been superceded by a mean of 16.5 yr, calculated from a larger group of observations. !Kung women may be anovulatory during some substantial portion of their birth intervals (mean length 35.4 months for all intervals observed during an eleven year period, $n = 102$) but they are not amenorrheic for this whole period.

It is tempting but premature to generalise from the !Kung to hunter-gatherers in general and hence to prehistoric populations. The low fertility of the !Kung suggests a stimulating hypothesis on the nature of population dynamics in such groups, as discussed by May, but exploring that hypothesis will require considerable additional research on other groups.

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The neural representation of visual space

DRASDO[1], and Lennie[2], attribute Rolls and Cowey[3] with the demonstration that monkey cortical magnification factor, M, varies as the square root of the ganglion cell density which deals with the corresponding region of visual field. Lennie's discussion is based on the development of this relationship for monkey and man. In fact, Rolls and Cowey report M to be proportional to ganglion cell density deg^2, not to its square root, both within and outside the foveal area.

Such proportionality between retinal ganglion cell density deg^2 and M has also been described at several stations of the visual pathways in a variety of species[4–7]. It has been a long-standing puzzle as to why a linear measurement, M, should be proportional to a retinal cell density.

The outcome of Drasdo's data manipulations, that human cortical magnification factor is proportional to the square root of the ganglion cells serving the corresponding region of visual field (that is, of his receptive field density, D_r), is thus not at all in agreement with the findings of Rolls and Cowey. If valid, it is the first instance of such a relationship and implies that the human visual cortex is organised in a quite different fashion from that of saimiri or macaque, or indeed from that of cat and rabbit.

However, the data on which Drasdo's conclusions are based are not sound enough to warrant a belief that the human cortex deviates so markedly from the organisation apparently common to a variety of species. The ganglion cell distributions which he pooled[8–10] were all obtained by the unreliable technique of counting on radial sections not subject to an Abercrombie correction[11]. Also, there is no evidence of common criteria for ganglion cells, shrinkages were not measured and the distributions plotted separately can support quite different interpretations from those of Drasdo. It perhaps suffices to point out that the results of Van Buren indicate densities of from one to more than two orders of magnitude greater than those of Oppel at the equivalent eccentricity. Correction for optical magnification is a nicety in such circumstances.

Of course, it would be pleasing if Drasdo's conclusions were valid, because the recent demonstration[12] of proportionality between human acuity and cortical magnification factor could then relate acuity directly to ganglion cell angular separation. In my opinion the evidence is against such a relationship in man[13] or cat[14] but, as Lennie points out, knowledge of the distribution of the specific ganglion cell class involved in resolution tasks may elucidate the matter. *Note added in proof:* Lennie's editorial remains explicitly misleading. This might have been avoided had Drasdo emphasised that he claimed differences between man and monkey which necessitated analysis in terms of $\sqrt{D_r}$, rather than D_r, and M. However, I remain unconvinced; the densities of Van Buren and Oppel differ more than 100-fold at the same eccentricity in the original data quite independent of errors introduced by replotting; similar large discrepancies occur throughout their data and preclude pooling.

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DRASDO REPLIES—Some responsibility is accepted for the scepticism evinced by Hughes, whose comments on my letter[1] can be traced to two irregularities on points of detail[2]. However, this scepticism should not extend to the material contents of the letter which can be shown to be unaffected. As Hughes indicates, the root sign in the first paragraph is regrettably misplaced. This relationship observed in animals was stated correctly in the following paragraph.

Apparent differences between the vision of man and other animals should not cause surprise. Even in other primates, predation, falls and fighting contribute largely to mortality[3]; survival may be related to peripheral and tectal vision, and correspondingly the human extra foveal retina has fewer presumed transient and feature-detecting ganglion cells[4].

The ganglion cell density distributions differed systematically; literally, to pool them would have been reprehensible. Instead, a mean line was taken between the two sets for each meridian (see Fig. 1, ref. 1). Shrinkage corrections for data of Oppel and Van Buren were based on fovea–papilla distance, which further reduces disparities by correcting for eye size. Paradoxically, Oppel's[5] original estimated total count fell short of Van Buren's[6] by only 40%, despite the extreme discrepancies referred to by Hughes.

I am indebted to Hughes, who inadvertently brought to my attention an incorrectly plotted point which, when corrected[2], reduced the discrepancy between cells and receptive fields in the area affected by excavation to 3%, giving further support to the calculation (see legend to Fig. 1, ref. 1).

The possibility of an overcount of ganglion cells is well known[6,7], but as with the other data used these were the best estimates available and all show an impressive alignment with the empirical equation which remains attractive for its simplicity and usefulness in the present state of knowledge.

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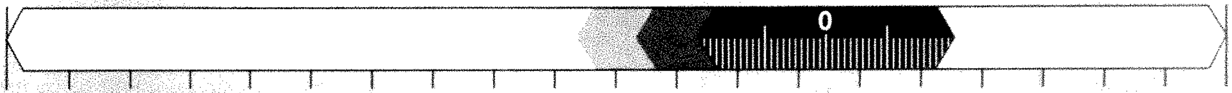

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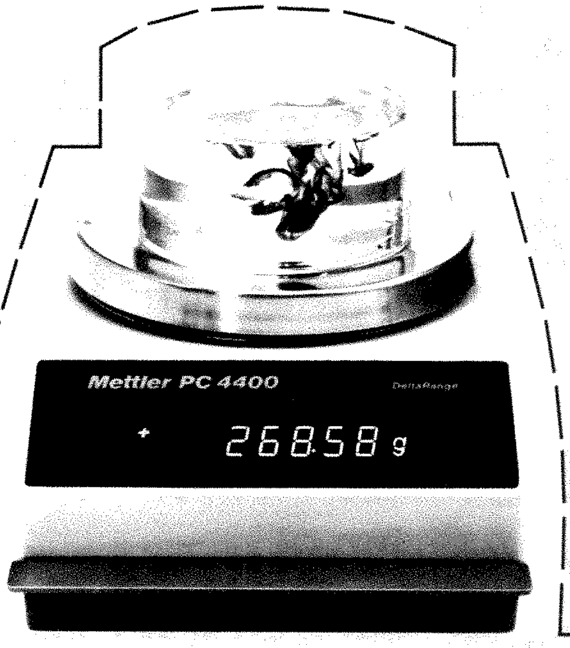

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The author describes the application of classical and modern experimental techniques to the study of the state and behaviour of metal ions in solution, and documents and reviews the resulting information. There are extensive tabulations of thermodynamic data relating to enthalpies and entropies of solvation, redox potentials, and hydrolysis and polymerisation equilibria. Concise yet comprehensive documentation of kinetic patterns brings out the common features and characteristics of the mechanisms of solvent exchange, complex formation and redox reactions. *(Ellis Horwood Series in Chemical Science)*

October 1978 482 pages
085312 027 7 $55.00/£25.00

Published by Ellis Horwood Ltd., Chichester, and distributed by John Wiley & Sons Ltd.

Advances in Modern Toxicology Vol. 5: Mutagenesis

edited by **W.G. Flamm**, *National Cancer Institute,* and **M.A. Mehlman**, *Mobil Oil Corporation.*

This discusses concepts in the field of mutagenesis and examines the potential magnitude of chemical mutagenesis as a public health problem. It tackles the difficult questions regarding permissible levels of exposure, procedures and approaches for quantifying risk, hazard or safety evaluation approaches. The magnitude of the mutagenicity problem and future research needs are outlined.

November 1978 414 pages
0470 99393 6 $35.00/£17.50

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Toxicology, Biochemistry and Pathology of Mycotoxins

edited by **K. Uraguchi**, *Tokyo University of Agriculture,* and **M. Yamazaki**, *Research Institute for Chemodynamics, Chiba, Japan.*

Experts in various areas of mycotoxin research present general reviews from the mycological, chemical, toxicological and pathological viewpoints.

November 1978 296 pages
0470 26423 3 $36.50/£19.50

Published by Kodansha Scientific Ltd., and distributed by John Wiley & Sons Ltd.

Cognitive Processes in Animal Behaviour

by **S.H. Hulse**, *Johns Hopkins University,* **H. Fowler**, *University of Pittsburgh,* and **W.K. Honig**, *Dalhousie University, Halifax, Nova Scotia.*

This volume highlights cognitive functioning in animals providing a link between research and theory on animal learning and behaviour and human learning and cognition. Although the contributors represent different fields of learning and behaviour, from ethological and experimental approaches, the book gives a cohesive and well-knit set of arguments that can provide a cross-fertilization of the techniques, strategies and conceptualizations used by both those doing research with humans and those using animals.

November 1978 480 pages
0470 26484 5 $42.00/£21.00

Published by Lawrence Erlbaum Associates and distributed by John Wiley & Sons Ltd.

Progress in Cybernetics and Systems Research Vol. III: General Systems Methodology, Fuzzy Mathematics & Fuzzy Systems, Biocybernetics and Theoretical Neurobiology

edited by **R. Trappl**, *University of Vienna Medical School,* **G.J. Klir**, *State University of New York, Binghampton,* and **L. Ricciardi**, *University of Salerno.*

Volume Three gives the latest results of research both in the theoretical development of cybernetics and systems research and in practical applications in several areas. Contributions are from outstanding scientists, from more than 25 countries.

November 1978 688 pages
0470 26371 7 $57.00/£28.50

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reviews

Chinese technical endeavour

N. Sivin

The Shorter Science and Civilisation in China. Vol. 1. An abridgement by C. A. Ronan of J. Needham's original text. Pp.326. (Cambridge University Press: London, New York and Melbourne, 1978.) £7.95.

For twenty-four years, in eight volumes of *Science and Civilisation in China* (still unfinished), several associated books, and a multitude of essays, Joseph Needham and his collaborators have been demonstrating the richness and importance of China's scientific and technical traditions before modern times. They have surveyed historical studies by hundreds of scholars in China, Japan and the West, supplemented them with explorations of their own, and woven them into a coherent whole for the first time. The assumption that Europe produced the only civilisation ever capable of systematically understanding and manipulating nature has been current too long to disappear merely because it has been massively disproven. But now that notion is a badge of wilful ignorance. To replace it Needham has offered his ecumenical vision of man's relationship to nature as the one area of experience in which the global effort has been common and convergent over the past several thousand years.

Because the intent of his work is "extensive and pioneering", Needham's books—even collections of essays and lectures such as *The Grand Titration* and *Clerks and Craftsmen in China and the West*—have been speculative in the best sense, closely argued, and massively documented. The latest volume of *Science and Civilisation in China*, a historical survey of alchemy, for instance, contains, alongside 262 pages of text, 169 pages of closely spaced bibliography. It brings the number of illustrations in the series so far to 1373.

After using Needham's publications in courses on Chinese science and medicine at MIT and the University of Pennsylvania for a dozen years, I can report that bright undergraduates with some experience in and taste for historical studies usually find these books readable and stimulating; that other students (especially science and engineering students, whose ability to learn outside their technical fields tends to atrophy in American universities) find them too dense; and that they are too expensive for students to own. The demand for a cheap, simplified version of Needham's survey, for students and others who want to live with the issues he raises, is therefore obvious. This book will satisfy it admirably.

The volume in hand covers Volumes I and II, on history, geography, the travel of scientific knowledge, and the history of philosophy, all seen from the special vantage point of science. Practically every aspect of Chinese technical endeavour since the New Stone Age is touched upon, with constant attention to corresponding aspects of other civilisations. This condensed version begins a new series of unspecified length.

Colin Ronan (who refers to Needham in the third person throughout) is an experienced author of books on science and its history for a general readership. He provides a straightforward condensation, with well-conceived minor rearrangements. He expresses himself clearly and simply, and preserves a good deal of Needham's nuance. The new version provides slightly over a third as much text, nearly as many tables, and more than half as many illustrations (some new), as the volumes abridged. The book is reasonably priced by current standards, and no doubt will become the first of Needham's books on China (in English, at least) to be published in paper covers.

The idea of a multi-volume abridgement is not intuitively obvious, but there is a great deal to be said for a level of detail that will put the gist of Needham on physics and physical technology, or on alchemy, protochemistry, and chemical technology, into one volume. Since the final fascicle of *Science and Civilisation in China* is nowhere in sight, the alternative to a serial abridgement would be an indefinite wait for a superficial single volume.

This book has several limitations of which its readers should be aware. Most important, it is based on writing roughly a quarter-century old. Needham's intellectual reach and the open-endedness of his arguments have made his writing less prone to obsolescence than most other Sinological surveys of the 1950s. His is still the only one that emphasises the scientific and technological components. Nevertheless, our understanding of many matters has been altered fundamentally by the burst of scholarship on China generally, and Chinese science particularly, in the past two decades.

Ronan has interpolated concise notes on important new discoveries and changes in chronology, and has corrected minor errors. But major re-writing, which would be the only way to incorporate important re-interpretations by Needham and many other scholars, is clearly not feasible. None of the investigators capable of fundamental re-writing — which would amount to a new synthesis of the field —is prepared to undertake it, and none known to me could produce a book as readable.

I find Ronan's condensation generally faithful. There are a few points where Needham's assertions are mashed beyond recognition—for example, to produce assertions that the Taoists favoured scientific logic (p83), that Shen Kua's late-eleventh-century account (publication misdated, p157) "is probably the first record in any language of the precipitation of metallic copper by iron", and that ancient Tantric Buddhists "adopted in their symbolism what we might call 'electrical energy' " (p271).

The bibliography is short, but not too short. It would have been more serviceable if the many mediocre and outmoded books had been omitted and more references to important introductory works and critical appraisals published in the past decade or two had been substituted for them.

Ronan's own view of the Chinese tradition, as set out in his brief introduction, puzzles me. To give only one example, the assertion that "before the Jesuit arrival Chinese science was quasi-empirical—based on observation and experience, with theory in a comparatively undeveloped state" ignores not only important parts of *Science and Civilisation in China* but many studies published by other historians in recent decades.

This and other puzzling notions in the introduction do not intrude upon the summary of Needham's arguments, but an introduction that explained the most important changes in our understanding in the past twenty years or so would have been a great help to readers.

All in all this first volume of *The Shorter Science and Civilisation in China* provides a splendid orientation to the cultural matrix of science and technology. It will motivate many readers to seek out more recent discoveries and interpretations. A large public will await Ronan's coming adaptations of Needham's histories of science, techniques and medicine. ☐

N. Sivin is Professor of Chinese Culture and of the History of Science at the University of Pennsylvania.

Electrical breakdown phenomena in gases

Electrical Breakdown of Gases. Edited by J. M. Meek and J. D. Craggs. Pp. 878. (Wiley: Chichester, UK, and New York, 1978.) £35; $75

THE book of the same title published in 1953 is well known as a standard reference book to engineers and physicists concerned with the many aspects of electrical breakdown phenomena in gases. This welcome new version, edited by the original authors, updates much of the material in the original volume and takes account of the many advances made over the past 25 years Inevitably the contents reflect changes of emphasis and new areas of current interest Discussion of lightning discharges and glow-to-arc transitions is now omitted but a chapter on laser induced breakdown has been included.

As in the original volume, chapter 1 is concerned with a discussion of the fundamental collision processes relevant to breakdown phenomena. This includes basic definitions and a description of electron scattering processes, negative ion formation, collisions involving molecular species, electron–ion and ion–ion recombination, and drift and diffusion of electrons and ions.

Chapter 2 is concerned with vacuum breakdown including a discussion of field emission, microdischarges and microparticle pre-breakdown phenomena. The discussion of spark breakdown in uniform fields in chapter 3 includes a full description of pre-breakdown ionisation in gases in steady-state conditions, electron avalanches, ionisation coefficients, and temporal growth of ionisation in impulse conditions.

A general treatment of corona discharges is given in chapter 4, and chapter 5 is concerned with spark breakdown in non-uniform fields. Breakdown voltage characteristics in both uniform and non-uniform fields are considered in chapter 6 for a wide range of gases and gas mixtures.

Chapter 7 deals with the time lag between the application of an impulse voltage and the consequent breakdown of a gas in terms of the so-called 'statistical time lag' and the formative time lags associated with the initiation and development of a discharge across a gap. In chapter 8, high frequency breakdown of gases is discussed in terms of radio and ultra high frequencies in air and sulphur hexafluoride

Laser induced electrical breakdown of gases is discussed in chapter 9. The intensity of a focused monochromatic beam is considered, together with its application to multiphoton absorption, ionisation and breakdown. Spark channels, which in recent years have become important in the design of opto-electronic devices of high sensitivity are considered in chapter 10. A short description of electrode effects is given in chapter 11.

Nine different well established researchers have contributed to this volume and they and the editors should be congratulated in achieving an unusual uniformity of style for a book of this type. Unnecessary overlap of material between chapters is minimal and the many excellent diagrams and illustrations throughout the book add to its attractiveness. It is a very readable book and should appeal to specialist researchers, postgraduate students, and readers whose interests lie only on the periphery of the general field of breakdown phenomena

H. B. Gilbody

H. B. Gilbody is Professor and Head of the Department of Pure and Applied Physics at The Queen's University of Belfast, Northern Ireland.

Liquid properties

Liquids and Their Properties: A Molecular and Macroscopic Treatise with Applications. By H. N. V. Temperley and D. H. Trevena. Pp.274. (Wiley: Chichester, UK and New York, 1978.) £15.

THE research work of the two authors of this book, Professor Temperley and Dr Trevena, lies, respectively, in the statistical mechanics of phase transitions and in the experimental study of the physical properties of liquids These fields exemplify the 'molecular' and the 'macroscopic' of the book's subtitle.

In the first field we have chapters 1–4 on intermolecular forces and statistical mechanics, chapter 10 on liquid structure and chapter 12 on transport properties. The rest of the book is mainly macroscopic: changes of phase, hydrodynamics and acoustics, liquids under pressure and tension (an interest shared by the authors), the gas-liquid surface, mixtures and solutions, and non-newtonian liquids. There is a final chapter on the unusual properties of liquid helium.

To cover this range of material in 270 pages allows only an elementary treatment The book aims to provide "what every good scientist, whether in research, teaching, industry or medicine should know about liquids." It is, perhaps, most successful in doing this on the macroscopic side, where the more descriptive narrative places less demands on the reader. The classical equations of hydrodynamics are derived painlessly with scarcely a result in vector notation that is not followed by its cartesian equivalent. Statistical mechanics is less easily handled this way and its equations have to be stated rather than derived if the book is to cover the wide range of subjects promised by the chapter titles.

Within these limitations the authors succeed in giving convincing and easily read accounts of their chosen topics. They are not entirely successful in the admittedly difficult job of relating the molecular side to the macroscopic. Moreover, some of the molecular theories discussed are now quite obsolete, such as the 'hole' and 'tunnel' theories of liquids and the lattice theories of liquid mixtures. There is a place in books of this kind for theories that are technically obsolete if they are physically informative (the authors rightly describe the theories of van der Waals), but not for theories which are now obsolete and which never did do justice to the basic physics. More recent and useful theories are, however, also described clearly, with particular emphasis on those put forward by the late Professor J. G. Kirkwood, to whom the authors rightly pay tribute.

The book is well produced and unusually free from misprints.

J. S. Rowlinson

J. S. Rowlinson is Professor of Physical Chemistry in the University of Oxford, UK.

Climate and Evolution

Ronald Pearson

November/December 1978, xii + 274pp., £14.00/$29.00 0.12.548250.7

One of the first synoptic treatments of two separate issues — the understanding of evolution and the problem of climate. It introduces the reader to information from a wide variety of relevant fields, and focuses attention upon the apparent synchroneity of past geophysical events, changes in the world's flora and fauna, and periods of historical importance. It will be of immediate interest to undergraduates and research workers in biology, climatology, geology and geography, and should encourage readers to appreciate the relevance of their data to an overall panoramic view of climate and evolution.

The Genetics and Biology of Drosophila

Volume 2b

edited by M. Ashburner and T. Wright

November 1978, xvi + 602pp., £29.60/$61.25 0.12.064941.1

Contents

J. Grossfield. Non-sexual behaviour of *Drosophila. L. Ehrman:* Sexual behaviour. *E. Gateff:* Malignant and benign neoplasms of *D. melanogaster. J. C. Sparrow:* Melanotic "tumours". *M. Ashburner and H. D. Berendes:* Puffing of polytene chromosomes. *T. M. Rizki:* The circulatory system and associated cells and tissues. *H. D. Berendes and M. Ashburner:* The salivary gland. *A. C. Crossley:* The morphology and development of the *Drosophila* muscular system. *T. M. Rizki:* Fat body. Author index. Subject index. Species index. Index of genetic variations.

The Genetics and Biology of Drosophila

Volume 2c

edited by M. Ashburner and T. Wright

December 1978/January 1979, xiv + 610pp., £32.00/$66.25 0.12.064942.X

Contents

A. Wessing and D. Eichelberg: Malpighian tubules, rectal papillae and excretion. *M. J. Lamb:* Ageing. *S. L. Fullilove and A. G. Jacobson:* Embryonic development — descriptive. *P. J. Bryant:* Pattern formation in imaginal discs. *N. M. Hodgkin and P. J. Bryant:* Scanning electron microscopy of the adult of *D. melanogaster. J. H. Postlethwait:* Clonal analysis of *Drosophila* cuticular patterns. *A. Shearn:* Mutational dissection of imaginal disc development. *W. J. Gehring:* Imaginal discs determination. *E. Hadorn:* Transdetermination. Author index. Subject index. Species index. Index of genetic variations.

Pesticide Microbiology

Microbiological Aspects of Pesticide Behaviour in the environment

edited by I. R. Hill and S. J. L. Wright

December 1978/January 1979, xx + 844pp., £38.00/$78.65 0.12.348650.5

This book presents the subject of pesticide microbiology in a balanced and authoritative way, neither minimizing nor over-emphasising the risks involved in our chemical interventions in the environment, and focusing attention on the diverse microbiological aspects of the fate and behaviour of pesticides. It describes how microbes can affect the activity and persistence of pesticides and how, in turn, pesticides can influence the activities of these members of the non-target biota. It recognises, too, that interactions with microbes cannot be considered in isolation from other (physical, chemical and biological) factors concerned with pesticide behaviour in the environment.

Biochemical Mechanisms of Liver Injury

edited by T. F. Slater

November/December 1978, approx. 850pp., £40.00/$82.75 0.12.649150.X

The book is primarily concerned with describing the variety of biochemical disturbances underlying a number of important liver injuries and diseases. Certain aspects in particular are emphasised, such as the role of metabolic activation of many hepatotoxins and hepatocarcinogens in the development of liver injury; the transient nature of such activated intermediates which makes rapid reaction studies necessary for their investigation; the importance of free radical reactions in the biochemical disturbances produced by these transient intermediates; and the multiplicity of protective mechanisms that may be applied. The book also includes some chapters dealing with the applications of relatively new techniques such as electron spin resonance, and nuclear magnetic resonance.

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Cosmic travelogue

Beyond the Moon. By P. Maffei. Pp. 376. (MIT Press: Cambridge, Massachusetts, and London, 1978.) $12.50; £8.75.

Beyond the Moon is a cosmic travelogue that takes us at ever-increasing velocities from the inner Solar System to the depths of the Universe. The purpose of adopting this unusual approach is to heighten the reader's awareness of the vastness of space. Some feel for the immensity of the heavens is conveyed by occasionally looking back at the Solar System until the Earth and Sun disappear from view. To adopt such a style of writing is a bold stroke: on the one hand it may captivate the curious reader, but there are points at which the description of the journey becomes altogether too precious and perhaps out of place in a scientific book.

Thus, on p167 we plunge into the cliché of holiday guide "Having witnessed the phastasmagorical spectacles of double stars and the cataclysms of novae and supernovae, we now set our sights on other goals . . .". Just round the corner to the Orion nebula: ". . . we tend at first to hold our breath, as if for fear that these tenuous veils will come undone . . . but the nebula remains . . . changeless in its very evanescence". The metaphor looks overworked even in the local stellar neighbourhood, where super-drive would be needed to dive through worm-holes of space in order to complete the itinerary.

For those willing to admit that suspension of disbelief necessary for enjoying science fiction, *Beyond the Moon* is a good read. It describes the Solar System, the nearest stars, and our Galaxy. Among the many commendable features is a surprisingly thorough survey of variable stars, a topic often skimped in popular writing. About one-third of the descriptive prose is a ramble through the extragalactic universe. Five appendices round off the journey, and there is also an outstanding index for those wishing to retrace their steps.

In no sense is *Beyond the Moon* a complete course in astronomy (neither author nor publisher claim that it is); rather it is like a series of extended essays in which the author is at his best when drawing out the themes that appeal to him particularly. His style and approach compares favourably with Asimov but lacks the incisiveness of Hoyle's writing at this same level.

Already *Beyond the Moon* has run through six editions in Italian, and it deserves to sell well in the English language. The translation and up-dating by D. J. K. O'Connell, S.J., of the Vatican, reads smoothly. Occasional lapses grate a little to anglophones (". . . the Protestant minister David Fabricius") and some analogy requires a good knowledge of Italian geography, such as knowing the distance by road from Rome to Genoa. This, however, is nit-picking, for O'Connell has done a fine job to this best-seller.

To whom is the book addressed? In the English language it must stand up to far more competitors than in its mother tongue. However, it seems admirably suited to that legendary and industrious hero of publishers' blurbs: the intelligent layman. It is a good account for the inquisitive but uninformed.

Simon Mitton

Simon Mitton is Secretary of the Institute of Astronomy, University of Cambridge, UK.

Structure of food webs

Food Webs and Niche Space. By J. E. Cohen. Pp. 189. (Princeton University Press: Princeton, New Jersey, 1978.) Hardback £9.40; paperback £4.65.

ECOLOGISTS have been surprisingly myopic about food webs. They have described them; complained about how complicated they are (the "tangled knitting hypothesis"); noticed that the number of trophic levels is never very many; perhaps commented on the fact that some seem to be compartmentalised; and then got on with the serious business of studying single-species populations, interspecific competition, species-frequency distributions and what have you. Questions about the shape of entire food webs within which the ecological play actually takes place, have hardly ever been asked.

In this book Cohen assembles data on thirty-one food webs, accumulated over four decades of field work by other biologists. He claims no first-hand knowledge of the natural history of the plants and beasts involved, but seems to have been meticulously careful in reading what others have written about them. He then analyses the structure of these food webs, focusing on patterns of food sharing. Specifically, he asks whether the overlap in food utilisation among various kinds of organisms can be represented in one dimension, or more formally as an interval graph. Cohen's central conclusion is that community food webs are interval far more often than chance alone would provide.

A great deal of the book is taken up with deciding what chance will provide if she is allowed to design random food webs—to generate the hypothetical tangled knitting as it were. Thus, after setting the scene and gently introducing his reader to the jargon of graph theory and combinatorics in chapters one and two (it sounds more difficult than it is) Cohen moves to a description of his real food-webs in chapter three.

Chapter four contains a major statistical analysis of the properties of these webs, and reveals a number of hitherto unexpected properties over and above the surprising fact that most, perhaps all real webs are interval. For example, the number of kinds of prey is roughly three-quarters the number of kinds of predators in all the community webs; the number of links between the bottom of a food chain and the top is highly constrained but has different distributions in different webs; and as a multiple of the number of predators in the food web, the distribution of dietary overlaps is roughly uniform. There is much food for thought in these, and the other empirical patterns revealed in this part of chapter four.

The remainder of chapter four and part of chapter five discuss a series of model worlds; seven random webs constructed under a variety of assumptions and constraints. (A large part of chapter five is also devoted to the clinical diagnosis of a sick, or at best perverse random number generator, and says much about Cohen's meticulous care in the analysis of both data and models.) The main conclusion from this, the core of the book, is very clear. The high frequency of interval webs in the real world is extremely unlikely to have arisen by chance.

The remainder of the book is devoted (in chapter six) to asking why food webs should be interval. Cohen runs through three minor, and three major possibilities. I must confess that of the three interesting hypotheses, I found one so vague, and one so esoteric mathematically that I did not understand them. The third, focusing on the notion that there are dynamic constraints on the design of food webs, is to my mind the most interesting. Cohen's use of qualitative stability analysis merely scratches the surface, and indeed may be misleading as a large number of webs that are qualitatively stable turn out not to be locally stable when parameters are assigned and the models analysed properly. There is much scope for further work here. Finally, chapter seven flies away into some fairly esoteric air—the higher corners of Euclidean space and intersection graph theory—which may keep the pure theorists happy but is unlikely to lead to many empirically testable hypotheses in the foreseeable future.

Obviously, the main empirical patterns which Cohen finds are only as good as the data on which they are based. Cohen himself is transparently honest about most of the weaknesses in his data, in the second part of chapter seven. There is no need to repeat them here. He also has some extremely perceptive remarks to make about data collection, and if the book does no more than encourage field biologists to collect new data on food webs that are specifically designed for and directed towards testing Cohen's hypotheses, it will have done a great service.

The main weakness in the data, however, is side-stepped by Cohen. In place of "species" he is often forced to use "kinds of organisms", and these may be very broad categories—for example, "spiders" feeding on "collembola" in Figure 1 Without wishing to nit-pick, there are actually rather a large number of species of collembola, and even more sorts of spiders even in the one Canadian willow forest to which this particular web refers, and they are not usually trivial components of terrestrial ecosystems. Thus, properly constructed, this and I would guess most of the other food webs might look totally different I do not know whether they would still be interval graphs, or how many of the other generalisations would still apply. This is decidedly not to say that Cohen should never have done the analysis in the first place. If ecologists always wait for perfect data nothing very interesting is going to get done Nor will people ever suspect that there are theoretical problems worth solving with better data But in this particular case we must definitely beware of the data, embrace the vision (Cohen claims that he got the idea in a dream), and take the book as a grand hypothesis More than anything else, I hope that Cohen's contribution will stimulate others to look more seriously at the design of foodwebs.

John H. Lawton

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Utilising phosphate resources

Phosphorus in the Environment. Its Chemistry and Biochemistry. Ciba Foundation Symposium chaired by R. J. P. Williams Pp. 320. (Elsevier: Amsterdam, 1978.) $31 75; Dfl.72 60.

THIS symposium report contains 16 papers given by a diverse group of experts on the utilisation of the world's phosphate resources The coverage is very wide; and chemical, biological, technical, ecological and economic factors are all discussed. Almost everything gets a mention. methods of birth control, water closets, marsh gas, nerve gas, toothpaste, detergents and even such mundane items as the current price of phosphate rock

Although the pure chemistry content is somewhat limited, the book abounds with statistics, predictions and speculations about the future of mankind and how it will be linked to the availability of phosphorus. Reserves, consumption and the eventual fate of the element as utilised by man are critically discussed at some length

Not unnaturally, there are differences of viewpoint and some contradictions in the data supplied by different authors. The symposium, nevertheless, makes a serious, valuable and timely contribution to its subject. Individual papers are of a high standard and the discussions are particularly informative

An obvious message emerging from the symposium is that fertilisers will always constitute the most important use for the element, and if a rapidly expanding world population is to be fed, the conservation of phosphate supplies is already a matter requiring serious attention

Phosphates are not in themselves harmful to life, and pollution problems arising from their use are local, often controllable, and are probably somewhat overrated.

With perhaps as much as three-quarters of the world's phosphate reserves lying in Arab countries, these reserves will undoubtedly, in the long term, prove to be their most valuable asset. Unlike oil, which can be replaced by alternative energy sources, phosphorus is irreplaceable in life systems

It is pointed out that the complete natural cycle of phosphorus, unlike those of other major life elements, probably extends over many millions of years. This may be associated with a natural depletion of dry-land deposits and a temporary build-up of phosphate on the ocean floor. It is argued by some that the sudden and rapid exploitation of phosphorus compounds during the past 100 years, and the continued increase in their rate of consumption, will in a century or so lead to exhaustion of dry-land phosphate rock supplies

It would therefore seem prudent not to worry unduly about detergents, but rather to concentrate on improving the present low efficiency of uptake of phosphorus from soils This might be done, either by modification of existing fertilisers and soil systems, or by genetic engineering of suitable new plant species.

The eventual exhaustion of dry-land deposits, if this is to be believed, may one day necessitate direct sea-bed phosphate mining Alternatively it may be possible to obtain food from suitable sea plants which could utilise phosphate directly from the ocean.

This is not a book for a newcomer, but it should appeal to all those with some previous knowledge of the subject who have wide interests in phosphorus science

D. E. C. Corbridge

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Microbial growth in extreme conditions

Microbial Life in Extreme Environments Edited by D. J Kushner. Pp. 465. (Academic. London and New York, 1978) $40 50; £19.60.

THIS book needed to be written in view of the increasing significance of microbial growth in extreme conditions. The extreme environmental conditions chosen are principally physical ones: extremes of temperature, pressure, pH value, water and salt activities and radiation. Heavy metal toxicities are also included This selection may be considered rather arbitrary, as it excludes the class of extreme environments caused by deficiencies of nutrients—for example, the trace elements, although excess of these elements is dealt with under toxicities.

A great interest of extreme environments is that the description of their effects may throw light on the effects of the normal or non-extreme environmental conditions. The special adaptations revealed may suggest possible new technological applications of the microbes. However, the main motivation of these studies has been to explain microbial ecology

Many of the articles tend to be catalogues of facts which are useful for reference but not compelling reading. Also there is much turgid description of effects due partly to a lack of use of the quantitative parameters of growth such as specific growth rate and lag period.

The state of knowledge in the field seems to be in an early descriptive phase. The development of general explanatory principles will require much more study in depth of the problems from a whole organism viewpoint as well as molecular study. The reader will find in this work the growing points of the subject as well as a valuable reference source.

S. J. Pirt

S J. Pirt is Head of the Microbiology Department at Queen Elizabeth College, University of London, UK.

obituary

James Craigie

PROFESSOR JAMES CRAIGIE, O.B.E., F.R.S., distinguished for his wide research in bacteriology, died in Edinburgh on 26 August 1978, aged 79.

He was born in 1899 and educated in Perth and at the University of St. Andrew's, where he qualified M.B., Ch.B. in 1923, and later Ph.D. and D.P.H. He was appointed assistant in bacteriology at the University in 1927. In 1931 he moved to the Connaught laboratories, University of Toronto, first as research associate, later as lecturer in epidemiology and, in 1946, Professor of Virus Infections. He returned to Britain in 1947 to join the staff of the Imperial Cancer Research Fund.

Craigie's introduction to microbiology was in the laboratories of St. Andrew's University under Professor W. J. Tulloch. Here he carried out work on the serology of typhoid and, with Tulloch, worked and published on the 'flocculation' reaction which occurred when vaccine lymph was mixed with vaccinia-immune serum. He continued these studies when he moved to Canada.

He showed that the 'flocculation' was due to a combination of agglutination of virus particles and precipitation of a soluble substance. Further studies with F. O. Wishart revealed that the precipitable substance consisted of two elements, one heat-labile (L), the other heat-stable (S). By ingenious techniques, separate antibodies against the L and S fractions were obtained; it became clear that a complex antigen (LS) containing both substances was present on the surface of the virus particle.

Following work over several years on the antigenic properties of typhoid bacilli, his most important contribution to knowledge was his work on typhoid bacteriophages. With K. F. Brandon he described in 1936 a phage specific for strains of *B. typhosus* (now called *Salmonella typhi*) containing what he described as Vi-agglutinogen. With C. H. Chen in 1938 he found other typhoid phages divisible into four types according to a range of properties. One of these (Type 2) developed a high selective lytic activity for the strain of *B. typhosus* on which it was propagated. Its activity could be regularly modified by changing the strain on which it was grown. With this research tool they found that typhoid bacilli could be divided into a number of types (24 were known by 1947). Strains which could be related on epidemiological grounds all fell into a single type. Here, then, was a method of great value in epidemiology, whereby evidence could be obtained as to whether individual infections could or could not be traced to a common source. The finding has had wide repercussions, for similar methods have been applied to the differentiation of strains of various salmonellas and other organisms.

During the Second World War Craigie was one of a number of workers concerned in developing a vaccine against epidemic typhus, and he also studied the serological relations of epidemic and murine typhus. He was a member of a joint United States-Canadian Commission which organised work on protection against rinderpest; it was feared that the enemy might try to introduce the disease into North America.

In 1946, W. E. Gye, the Director of the Imperial Cancer Research Fund, was touring North America to learn of developments in the cancer field, and Craigie accompanied him. Gye then persuaded him to return to Britain to the Fund's laboratories at Mill Hill with a view to becoming Director; this he did, succeeding Gye in 1949.

Gye, always a passionate believer in the virus theory of cancer held that the survival of mammalian tumour cells after freezing or drying indicated that cellular survival could be ruled out, and that a virus must be concerned. Craigie, however, showed that in frozen tissues, some particularly resistant cells might survive in a modified, 'paramorphic' state. He designed a very effective tissue mincer, and, more important, a cabinet for preserving transmissible tumours at -70 °C. As a result, numerous propagable tumours, especially those of mice, could be preserved for years; no longer was it necessary to passage them *in vivo* at frequent intervals, and much time and money were saved. His last work was on Tyzzer's disease of mice, a very troublesome infection in mouse colonies; he was able to cultivate the causative organism (*B. piliformis*) in fertile eggs.

Craigie was not really happy as Director, preferring to concentrate on his own research and in 1957 he was allowed to do this, Dr R. J. C. Harris assuming overall responsibility for directing the Mill Hill laboratory.

In 1929 Craigie married Margaret Fotheringham, who predeceased him. He is survived by two daughters. He was elected F.R.S., Canada, in 1946 and F.R.S., London, in the following year.

He will be especially remembered for the leading role he played in Canadian microbiological research, especially in the years before and during the war, when work on viruses was developing so rapidly.

C. H. Andrewes

Harold Fletcher

DR HAROLD ROY FLETCHER, Queen's Botanist in Scotland, Regius Keeper of the Royal Botanic Garden, Edinburgh, from 1956 to 1970 died in Edinburgh on 27 August 1978. Born in Glossop, Derbyshire, he studied under Professors Weiss and Lang in Manchester and then moved to the Department of Botany of the University of Aberdeen as assistant to Professor Craib.

In Aberdeen his first responsibility—to his later amusement—was to teach plant physiology, but he soon turned to his main scientific interest, plant taxonomy and gained a PhD in 1933 for work on the Verbenaceae of Siam. At the same time he had a taste of plant collecting in the Faroes and Iceland and experience of the montane flora of Scotland for which he had an abiding affection.

In 1934 he moved to the Royal Botanic Garden, Edinburgh, then under the direction of Sir Willian Wright Smith and continued work on Asiatic floras gaining a DSc from Edinburgh University in 1939. Then followed a most rewarding period working on the rich collections of Chinese plants including a monograph of the genus *Primula* in collaboration with Sir William. At the same time he taught many university courses in botany, especially a course in taxonomy, which in its modern synthetic approach had no equal in Britain. Several strong modern currents in plant taxonomy in Britain flowered from these lectures.

During the years 1951–54 he turned his attention to horticulture—he had a rare eye for 'a good plant'—as Director the Royal Horticultural Society's Garden at Wisley in Surrey. And from this stemmed his activity in horticultural nomenclature as secretary for ten years of the International Commission for Horticultural Nomenclature and Registration and his detailed enumeration of Rhododendron cultivars —all recognised by his award of the Royal Horticultural Society's Victoria Medal of Honour in 1956.

A year after his return to the Edinburgh Garden in 1955 he succeeded Wright Smith as Regius Keeper. Immediately his energy and enthusiasm were thrown into rejuvenating botany and horticulture in the Garden, in Edinburgh, in Scotland and further afield. He relentlessly badgered authority to give him the necessary resources and so built a new herbarium and library in 1964 and new glasshouses in 1967. The staff was enlarged and better equipped, the outlook in taxonomy and horticulture remoulded and the training of horticulturists recast.

His continuing interest in botany in Scotland found an outlet in stimulating the post-war revival of the Botanical Society of Edinburgh but his much wider interest in world botany led to his advocacy of Edinburgh as the site of the Tenth International Botanical Congress. For this Congress in 1964 he shouldered much of the planning as general secretary. This Congress placed botany in Edinburgh before a wide audience, but in addition by travels and lectures, especially in North America, he formed strong personal links worldwide—especially as President of the International Association of Botanical Gardens 1964–69.

His outstanding devotion to Botany in Scotland was recognised by his much cherished appointment as Queen's Botanist in Scotland in 1967, as an Honorary Professor of Botany in the University of Edinburgh in 1968 and by the award of honorary DSc's by Edinburgh in 1971 and St Andrews in 1972 and the Patrick Neill Prize by the Royal Society of Edinburgh 1971–73.

His interest in writing and horticultural and botanical history found an outlet in his *Story of the Royal Horticultural Society 1804–1968* in 1969 and (with W. H. Brown) *History of the Royal Botanic Garden, Edinburgh 1670–1970* published in time for the garden's tercentenary celebrations. Following his retirement in 1970 he wrote a detailed account of the plant collecting travels of Ludlow and Sherriff—*A Quest of Flowers*.

Throughout his professional life he had a deep love of the arts especially literature, music and painting of which he had a discriminating collection. In his retirement he found more time for these pursuits and served as Chairman and then as a member of the Governors of the Edinburgh College of Art.

Harold Fletcher was possessed of a most remarkable blend of science and the arts intermingled with a strong human compassion. His taxonomy was science tinged with art, his administration science guided by humanity Throughout his life he drove himself hard and was loyally and very knowledgeably supported and encouraged by his wife Betty and by his son and daughter.

D. M. Henderson

John Marwick

DR JOHN MARWICK, the leading palaeontologist in New Zealand during the middle decades of the twentieth century, died in Hawkes Bay, New Zealand, on 16 August 1978, at the age of 87.

The son of an Orkney wheelwright who migrated to Otago in the eighteen-eighties, Marwick was educated at Oamaru, attending Waitaki Boys' High School, where the rector, Dr R. H. Don, was changing the bias of the curriculum from classical to scientific, stimulating an interest in geology by classes and excursions such as were seldom available to secondary school boys. Entering the teaching profession, Marwick attended Otago University, graduating M A. with first class honours in geology, and winning the Ulrich Memorial Medal in mineralogy and petrography, thereafter teaching in South Canterbury for two years before the outbreak of World War I. In the Middle East (1916–19) he served in the Camel Corps and N Z. Mounted Field Ambulance, was twice wounded and was awarded the Military Medal.

On his return from the war, Marwick was appointed to work in palaeontology at the New Zealand Geological Survey (then under the Mines Department, but later a division of the Department of Scientific and Industrial Research). His research on Tertiary molluscs led in 1924 to the award of D Sc. (University of New Zealand) for a revision of New Zealand Veneridae (Bivalvia).

Between 1921 and 1976 he published about 125 papers, reviews, and bulletins, his research continuing for 20 years after his official retirement from the Geological Survey (as Chief Palaeontologist) in 1952 He brought a sound objective judgement to the study of the systematics and biostratigraphy of New Zealand Bivalvia and Gastropoda. His revisions (illustrated by his own pen and ink drawings) of Struthiolariidae, Naticidae, Volutidae, Veneridae, Cardiidae and his monographs of Tertiary faunas, notably the Danian (Palaeocene) Wangaloan fauna, described jointly with the late Dr H J. Finlay, earned John Marwick a world reputation as a student of fossil Mollusca

With his colleague Finlay, who pioneered study of New Zealand Cretaceous and Cenozoic Foraminifera at the Geological Survey, Marwick was also responsible for defining and improving the standard sequence of local biostratigraphic stages that have been of great value in geological mapping in New Zealand As a primarily Cenozoic palaeontologist, he regarded his occasional deviations into the Mesozoic as somewhat amateurish, yet his contributions include a standard reference work on New Zealand Triassic and Jurassic fossils On the other hand he welcomed field work in a Geological Survey faced by the hard times and stringencies of the great economic depression, when Marwick was expected to assist in geological mapping and writing diverse reports with little relevance to palaeontology He gained great satisfaction from descriptive geomorphology and stratigraphy, so that his publications in these subjects, for instance on the physiography and structure of the Rotorua–Taupo volcanic zone (with H.E. Fyfe) and on the geology of Te Kuiti district must always rank high among his achievements.

Dr Marwick might well have become Director of the Geological Survey, but although he served as Acting-Director on occasion, he preferred to remain in his palaeontology laboratory. On several occasions he lectured in geology at Victoria University of Wellington (then a college of the University of New Zealand) when his close friend Dr C. A. Cotton (later Sir Charles), well known for his geomorphology text books, was overseas on sabbatical leave; and he also served as external examiner for higher degrees He took an active part in the Royal Society of New Zealand, which had awarded him the Hamilton Prize for beginners in science (1926) and elected him a Fellow in 1935; he was awarded both the Hector and the Hutton Memorial Medals of the Society for his contributions to palaeontology. He served on Council, was Honorary Editor of the Society's *Transactions* (1938–46) and was elected Vice-President (1950–52) but declined nomination as President.

Especially during the search for petroleum in New Zealand between the wars and after 1945, Marwick introduced many overseas geologists to New Zealand stratigraphy and he recalled with pleasure his excursions to fossil localities with Drs T. Wayland Vaughan, Axel A. Olsson, F. A. Singleton, and other visiting palaeontologists.

Jack Marwick was an unselfish man of high integrity, whose humanity and altruism made him a father figure to the many young geologists he helped in their careers; while his sincerity, good humour, and broad-based culture earned the friendship and respect of his colleagues.

C. A. Fleming

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STODDART, D R, and JOHANNES, R E. (edited by). Coral Reefs: Research Methods (Monographs on Oceanographic Methodology, Vol 5) Pp.xv+581 ISBN-92-3-101491-9. (Paris. Unesco, 1978.) np

THIEDE, Walther British and European Birds. Illustrated with Colour Photographs. Translated by Annette Brandon (Chatto Nature Guides) Pp 144 ISBN-0-7011-2322-2. (London Chatto and Windus, Ltd, Toronto Clarke, Irwin and Co. Ltd, 1978.) £1 95 net

TRESEDER, Neil G. Magnolias Pp xviii+243. ISBN-0-571-09619-0. (London and Boston, Mass Faber and Faber, 1978 Published in collaboration with the Royal Horticultural Society) £30 net.

WALLACE, Robert A Biology The World of Life Second edition. Pp xvi+584. ISBN-0-87620-109-5. (Santa Monica, CA Goodyear Publishing Company, Inc; Hemel Hempstead, Herts Prentice-Hall International, 1978.) £11 65

WATERLOW, J C, GARLICK, P. J, and MILLWARD, D J Protein Turnover in Mammalian Tissues and in the Whole Body. Pp viii+804. ISBN-0-444-80021-2. (Amsterdam, New York and Oxford North-Holland Publishing Company, 1978) Dfl. 276, $120.

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UNIVERSITY COLLEGE CARDIFF

Applications are invited for the post of

GRADUATE RESEARCH ASSISTANT

(1 year contract) jointly in the DEPARTMENT OF APPLIED MATHEMATICS AND ASTRONOMY and the DEPARTMENT OF BIOCHEMISTRY. Duties to commence as soon as possible.

The post is suitable for a graduate in Chemistry, Biochemistry or related discipline and the work will be in collaboration with Dr A H. Olavesen, Sir Fred Hoyle and Professor N. C Wickramasinghe on Laboratory Studies of the Infrared Spectra of Interstellar Polymers.

Applications (2 copies), together with the names and addresses of two referees, should be forwarded to the Vice-Principal (Administration) and Registrar, University College, P O. Box 78, Cardiff CF1 1XL. Closing date January 31, 1979 Please quote reference 1644. 8549(A)

ROTHAMSTED EXPERIMENTAL STATION

Harpenden, Herts. AL5 2JQ

SENIOR PRINCIPAL SCIENTIFIC OFFICER

to assist in the supervision of work in the Soils and Plant Nutrition Department, especially of projects with a physico-chemical or mathematical bias

Qualifications should include a good basic training in physical chemistry or other physical science, with a distinguished record of work in a topic related to soil science.

Appointment in the grade of Senior Principal Scientific Officer on a scale rising by two annual increments from £10,043 to £11,300 p.a. Non-contributory superannuation

Apply in writing to the Secretary giving names and addresses of two referees and quoting Ref 364 by December 22, 1978. Further details on request. 8547(A)

POSTDOCTORAL POSITION

in development of field desorption double focusing mass spectrometry and associated techniques, and applications to studies of polar, labile biological substances. Experience in physical, instrumental and computer aspects as well as working knowledge of structural studies in bio-organic chemistry necessary. Position available for 1 year with possibility for renewal based upon performance Salary $11,880 to 14,916 Send curriculum vitae, names of three references and reprints/preprints to: Dr A L Burlingame, Biomedical Mass Spectrometry Resource, Space Sciences Laboratory, University of California, Berkeley, Ca. 94720. The University of California is an Equal Opportunity Employer. 8537(A)

INSTITUTE OF TERRESTRIAL ECOLOGY

HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER

CHEMIST

A chemist at Higher Scientific Officer/Senior Scientific Officer level is required to join a team concerned with research on the fate and effects of atmospheric pollutants. The team, which is studying forest ecosystems, needs a greater understanding of chemical events occurring on leaf surfaces, including surface exchange processes and the release of foliar leachates.

The successful applicant will be based at the Institute's Bush Estate Station, seven miles south of Edinburgh.

Qualifications

Candidates should possess a good 1st or 2nd class honours degree or its equivalent in chemistry and have at least two years' post graduate research experience. A specialist interest in physical chemistry or biochemistry would be an advantage.

Salary and Conditions of Service

The salary will be within the following scales.

H.S.O. £4,101 to £5,448 per annum; S.S.O. £5,154 to £6,898 per annum with 22 days paid annual leave plus 10½ days Public and Privilege holidays

Starting pay according to age, qualifications and experience.

There is a non-contributory superannuation scheme.

The Natural Environment Research Council is not a Government Department but conditions of service are similar to those of the Civil Service.

Application forms and further details are available from:

Establishments Section
Institute of Terrestrial Ecology
68 Hills Road
Cambridge
CB2 1LA (0223 69745)

Closing date for completed applications is December 8, 1978.

Please quote reference 363.

8511(A)

EAST MALLING RESEARCH STATION

FRUIT BREEDING DEPARTMENT

PLAN BREEDER/ GENETICIST

to join team breeding new varieties of fruit crops.

Applications are invited from graduates with First or Upper Second Class Honours degree in Botany with Plant Genetics.

Appointment Scientific Officer grade (£2,839 to £4,415) or Higher Scientific Officer grade (£4,101 to £5,448), at least two years post qualifying experience is required for appointment as Higher Scientific Officer.

Details and application form from Assistant to the Secretary, East Malling Research Station, East Malling, Maidstone, Kent ME19 6BJ. 8535(A)

INSTITUTE OF TERRESTRIAL ECOLOGY

HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER

EXPERIMENTAL PLANT ECOLOGIST

An experimental plant ecologist at Higher Scientific/Senior Scientific Officer level is required at the Institute's station at Bangor, Gwynedd. He/She will be expected to work with other scientific staff stimulating additional interest in a programme of vegetation management with grazing animals

Qualifications

Applicants should have a good 1st or 2nd honours degree or equivalent in agriculture ecology or a related biological subject and at least 2 years postgraduate research experience showing proficiency in quantitative ecology.

Salary and conditions of service

The salary will be within the following scales:

H S.O. £4,101 to £5,448 per annum
S S O. £5,154 to £6,898 per annum

with 22 days paid annual leave plus 10½ days Public and Privilege holidays

Starting pay according to age, qualifications and experience. There is a non-contributory superannuation scheme

The Natural Environment Research Council is not a Government Department but conditions of service are similar to those of the Civil Service.

Application forms and further details are available from:-

Establishments Section
Institute of Terrestrial Ecology
68 Hills Road
Cambridge
CB2 1LA (0223 69745)

Please quote reference 159.

Closing date for applications: December 8, 1978.

NATURAL ENVIRONMENT RESEARCH COUNCIL

8508(A)

ICI/UNIVERSITY OF LEICESTER

Joint Laboratory in Molecular Genetics

The above laboratory has vacancies for experienced post-doctoral molecular biologists and microbial geneticists and for grade 3 to grade 6 laboratory technicians to work:

(i) in the field of eukaryotic gene expression in prokaryotes, and
(ii) on the genetics and related biochemistry of the methylotrophs.

Appointments will be made for periods of up to 5 years, with salaries in the range:

£3,883 to £6,555 for post-doctoral Research Associates and £2,688 to £4,365 for technicians.

Salary scales are currently under review.

Applicants should send a detailed curriculum vitae and names of two references to both:

Professor W. J. Brammar,
Department of Biochemistry,
University of Leicester,
University Road,
Leicester LE1 7RH

and

Bioscience Group,
ICI Corporate Laboratory,
P.O. Box 11,
The Heath, Runcorn,
Cheshire WA7 4QE

8528(A)

Senior Scientist Metabolic Studies

This appointment within the Drug Metabolism section of our Research Department based in Nottingham, arises from internal promotion

It calls for someone capable of directing an established team and to be responsible for all in-vitro and in-vivo metabolic studies in animals and man, using radio-tracer techniques to investigate absorption, disposition and biological transformation of compounds intended for use in human medicine. The work also involves collaboration with staff working in other areas of the Research Department so the ability to communicate effectively is required

Candidates (men or women) must be graduates in biochemistry, chemistry or an allied scientific discipline and hold a higher degree in an appropriate field of research. Several years' experience in drug metabolism studies including extensive use of radio labelled compounds is essential.

Benefits include a profit sharing scheme and staff purchase privileges. Generous help with relocation to the area is available

Please write for an application form to. T. W. Flower, Employment Manager (Technical),

The Boots Company Limited,
Station Street, Nottingham, NG2 3AA.

8559(A)

MEAT RESEARCH INSTITUTE

PUBLIC RELATIONS OFFICER

A scientist of wide interests with a flair for communications work is required to present the research activities of the Institute to the world at large.

The preparation of a variety of articles for publication in journals, trade magazines and the popular press, assistance in the production of radio and television programmes, and organisation of exhibitions are a few examples of the range of duties In addition, the successful applicant will have a supervisory responsibility for the library service and for reception of visitors Further details are available on application

Qualifications: Science degree, H N C. or equivalent qualification with relevant experience or an honours degree. At least 5 years' relevant post-qualifying experience is required for appointment as H.S O Evidence of ability to write good English will be required. A knowledge of French and/or German would be an advantage.

Salary: S.O grade (£2,839 to £4,415) or H.S.O grade (£4,101 to £5,448), starting salary depending on experience.

Non contributory superannuation.

Closing date for applications: December 7, 1978

Application forms and further details obtainable from:

Personnel Officer
Meat Research Institute
Langford
Bristol
BS18 7DY.

8514(A)

UNIVERSITY OF NAIROBI KENYA

Applications are invited for the post of

SENIOR LECTURER

in the DEPARTMENT OF BOTANY.

Applicants should be qualified in any of the following disciplines of Genetics: Molecular Genetics, Population Genetics and Quantitative Genetics. In addition candidates must have a Ph D. in Botany with extensive teaching and research experience as evidenced by published work. The appointee will be expected to take part in the teaching of both undergraduate and postgraduate courses and to develop vigorous research programmes in his/her areas of specialisation. Further details may be obtained from the Chairman of the Department. Salary K£2,988 to £3,984 p.a. (K£1=£1 36 sterling). The British Government may supplement salary by £4,770 p a (sterling) for married appointee or £3,252 p a. (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages, S S.S.F. or F.S.S.U ; non-contributory medical scheme, subsidised housing/housing allowance

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Registrar (Recruitment and Training) University of Nairobi, P.O Box 30197, Nairobi, Kenya, by December 21, 1978. Applicants resident in the U.K should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

8558(A)

UNIVERSITY OF SURREY

DEPARTMENT OF HUMAN BIOLOGY AND HEALTH

EXPERIENCED TECHNICIANS GRADE 5

(£3,186 to £3,720) under review

Due to an expansion we require qualified and experienced technicians to join our existing team of twelve. The Department runs undergraduate courses in Human Biology and in Nursing Studies. The laboratories teach physiology, anatomy and related subjects in human biology The technicians will help with class preparation and also assist in one of the following areas of research:

- Neurophysiology, both human and animal
- Environmental physiology, using a new environmental chamber
- Biochemical and immunological research
- Studies of factors affecting human performance.

If you have an H N.C. in Applied Biology, Advanced City & Guilds Laboratory Technicians Certificate or similar, and have several years' laboratory experience (even if not directly in the above fields) we would like to hear from you

The department is in a new building in attractive surroundings, adjacent to an historic town with excellent recreational facilities on the campus and in the surrounding area

Further particulars and application forms for the posts may be obtained from the Staff Officer, University of Surrey, Guildford, Surrey GU2 5XH or telephone Guildford 71281, ext 452.

8572(A)

INSTITUTE OF TERRESTRIAL ECOLOG

HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER

RADIO-CHEMIST

A radio-chemist is required, Higher Scientific Officer/Senior Sc tific Officer level, to work in conj tion with ecologists on the reten and movement of radionuclides semi-natural terrestrial ecosystems.

Applicants will be expected to ticipate in the planning of the resea programmes, data assessment and paration of reports in co-operat with the ecological staff.

The successful officer will be be at the Institute's Merlewood Resea Station, Grange over Sands, Cumbr

Qualifications and experience

Candidates should possess a 1st 2nd class honours degree or equiva in Chemistry, Physics or related ject and have at least two years' r vant post graduate experience.

Salary/conditions of service

Starting salary according to qualifications and experience on following scale

£4,101 to £6,898 per annum with days paid annual leave plus 10½ Public and Privilege holidays Ther a non-contributory superannua scheme.

The Natural Environment Resea Council is not a Government Dep ment but conditions of service similar to those of the Civil Servic

Application forms and furt details are available from.

Establishments Section
Institute of Terrestrial Ecolog
68 Hills Road
Cambridge
(0223 69745)

Please quote reference 362.

The closing date for comple applications is: December 8, 1978.

8512(A

THE UNIVERSITY OF NEWCASTLE

Newcastle, N S.W., Australia

POSTDOCTORAL RESEARCH FELLOW

DEPARTMENT OF MATHEMATICS

Field: Renormalisation Gr Theory in Statistical Mechanics.

Project: The successful candi will be familiar with the theory Renormalisation Group methods their application to Crit Phenomena, and will work on a pro entitled "Investigation of translationally invariant lattice mo by Renormalisation Group Perturbation Expansion methods". this project a study will be made magnetic surfaces and films, poly films and surfaces, percolation a surface and in confined geomet and related problems.

Qualifications: A Ph D. or equivalent, preferably with some p doctoral experience in the appropr field.

Salary. Australian $A13,343 $A16,882, plus a travel allowance up to $A2,000

Tenure. The appointment will for one year in the first instance, the prospect of renewal for two fur years

Further scientific details of project may be obtained from Head of Department, Department Mathematics, University of Newca N S W , Australia, (Assoc Prof. J Guttmann)

Applications giving relevant sonal and professional details, and names of at least three referees sh be sent to:

Vice-Principal,
University of Newcastle,
Newcastle, N.S.W. 2308;
AUSTRALIA.

8541(

BERKELEY PHYSICS

The Physics Department of the Uni- rsity of California, Berkeley, expects ring the next two years to be mak- g one or two appointments at the sistant professor level. Preferred areas research are astrophysics, atomic d plasma physics, condensed matter ysics, nuclear physics, and particle ysics, with some priority given to eoretical condensed matter physics, perimental plasma physics, and eoretical particle physics.

Applicants should write before ebruary 1, 1979, to the **Chairman of e Physics Department, University of alifornia, Berkeley, CA 94720, USA,** ving curriculum vitae, publication st, and references. The University of alifornia is an Affirmative Action mployer. 8569(A)

AGRICULTURAL RESEARCH COUNCIL

LETCOMBE LABORATORY

Scientific Officer/Higher Scientific fficer required in the Soil Atmos- ere and Nitrogen Section of the eld Studies Department of Letcombe boratory.

The Section is concerned with the te of nitrogen fertilizer and factors ecting its uses for grasslands and real crops under a range of cultiva- n techniques. The officer appointed ll be responsible for planning and ecuting part of this research and r the development of new experi- ental approaches to understanding e fate of nitrogen fertilizer.

Minimum qualifications: First or per second class honours degree or equivalent qualification with at st two years relevant post qualifying perience for appointment at the gher grade.

Salary scale for Scientific Officer ,839 to £4,415 and Higher Scientific ficer £4,101 to £5,448. Non- ntributory superannuation scheme.

Appointment at Senior Scientific ficer (£5,154 to £6,898) level would considered for an exceptionally well alified candidate.

Application forms and further tails from The Secretary, Agricul- al Research Council, Letcombe boratory, Wantage, Oxon OX12 T by December 7, 1978. 8515(A)

Central Birmingham Health District

IRMINGHAM MATERNITY HOSPITAL

CYTOGENETICIST

Trere is an immediate vacancy for experienced Cytogeneticist (Basic ade Scientific Officer) in the Infant velopment Unit at the above hos- al. The laboratory is one of two ponsible for the service needs in West Midlands Region. There is taff of four full time Cytogeneticists, ticipating in all aspects of the work there is a possibility of promotion. plicants should hold a relevant ence degree.

Salary scale £3,486 rising to a ximum of £4,899.

Visits to the laboratory may be anged by appointment with Dr meron, Tel 021-454 4851, Ext. 224 ildrens' Hospital).

Application forms and job descrip- ns from Personnel Officer, Birming- n Maternity Hospital, Edgbaston, mingham B15 2TG. Tel 021-472 7. 8554(A)

UNIOR TECHNICIAN required in Division of Molecular Biology at Institute of Cancer Research boratory, Fulham Road, London 3, to assist with work on the pro- s of the genetic material. Candi- es should have at least two 'A' els in Chemistry, Physics, Maths or logy. The appointment is for a ee year period initially. Salary in le £2,169 per annum to £3,015 per um according to age and qualifi- ions, plus London Allowance of 4 per annum. Applications in dupli- e with the names of two referees to Secretary, Institute of Cancer earch, 34 Sumner Place, London 7 3NU, quoting ref. 301/B/55. 8527(A)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

CLINICAL SCIENCES BUILDING,
ROYAL FREE HOSPITAL,
HAMPSTEAD, LONDON

SENIOR LECTURESHIP IN PHYSIOLOGY

Applications are invited for the above post which is available from January 1, 1979. Duties will include teaching and research. The Department of Physiology is now housed in new premises in Hampstead which are integral with the Royal Free Hospital, giving improved facilities for joint work and cooperation with clinical departments. All departmental activities take place in the new premises except for occasional lectures and meetings in the Hunter Street section of the School. Medical qualifications will be an advantage but are not essential. Salary will be on the preclinical scale in the range £7,506 to £9,263 plus £450 London Allowance.

Arrangements to visit the department should be made directly with Professor C. B. B. Downman (tel. 01-794 0500, ext. 4301). 6 copies of applications (preferably typewritten) giving full details with names and addresses of two referees should be sent by December 15, 1978 to the Secretary, Royal Free Hospital School of Medicine, 8 Hunter Street, London WC1N 1BP, from whom further particulars may be obtained. 8543(A)

HOUGHTON POULTRY RESEARCH STATION

GENETICIST

A vacancy exists in the Leukosis Experimental Unit for a geneticist to study the inheritance and nature of resistance of chickens to oncogenic and other viruses, and to assist in the formulation of breeding programmes for the Institute's flocks.

Applicants must have a first or upper second class honours degree, with genetics as a major subject in the first or a higher degree, and preferably a Ph.D. or at least 3 years' relevant postgraduate experience. A minimum of 4 years' relevant postgraduate experience is required for the S.S.O. grade.

The appointment will be to the Higher Scientific Officer grade (£4,101 by 8 to £5,448) or the Senior Scientific Officer grade (£5,154 by 8 to £6,898), grade and starting salary depending upon experience. Non-contributory superannuation scheme.

Further particulars and application forms from the Station Secretary, Houghton Poultry Research Station, Houghton, Huntingdon, Cambs. PE17 2DA. Closing date for applications is January 10, 1979. 8507(A)

UNIVERSITY OF LONDON KING'S COLLEGE

DEPARTMENT OF BIOCHEMISTRY

RESEARCH ASSISTANT

Applications are invited for the position of Research Assistant to work upon the catabolism of abnormal haemoglobins in rabbit reticulocytes and erythrocytes. The post is available for three years, financed by a grant from the Wellcome Trust and the salary will be £3,384 per annum plus £450 per annum London Allowance, with annual increments on the scale £3,384 to £3,883 per annum, plus £450 per annum London Allowance. Universities Superannuation Scheme contributions would be payable.

Candidates should have a good Honours Degree in biochemistry or a related subject and research experience.

Applications, together with the names of two referees, should be sent to Dr A. R. Hipkiss, Department of Biochemistry, Strand, London WC2R 2LS to reach him no later than Friday, December 1, 1978 quoting reference **191652/N.** 8540(A)

There is a vacancy for a BIOLOGIST in *Nature's* London office. He or she would join the team which assesses the suitability of biological manuscripts for publication; there would also be opportunities to work with members of the *Nature* staff in compiling other sections of the journal. The job might appeal to the holder either of a bachelor's or an advanced degree.

Write to:
The Editor
NATURE
MACMILLAN JOURNALS LIMITED
4 Little Essex Street
London WC2R 3LF

enclosing a curriculum vitae and any other material you consider relevant.

8577(A)

The University of British Columbia

DEPARTMENT OF CHEMISTRY

Applications are invited from candidates with teaching and research interest in any aspect of INORGANIC CHEMISTRY including BIOLOGICAL INORGANIC CHEMISTRY for an appointment at the Assistant Professorship level.

Applications consisting of a curriculum vitae, a list of publications and the names and addresses of three referees should be sent to the undersigned before December 31, 1978. This post is open to both male and female applicants.

Professor Charles A. McDowell
Department of Chemistry
University of British Columbia
2075 Wesbrook Mall
Vancouver, British Columbia
Canada V6T 1W5

8519(A)

Botswana

Wildlife Conservation Officer (Training)

Up to £8,850 plus allowances

The successful candidate will be responsible for the development, administration and implementation of training programmes concerning the conservation, management and control of wildlife in Botswana. He or she will also be expected to develop a detailed course syllabus suitable for the various levels of courses run by the Wildlife Training Centre.

Candidates should possess a degree in Zoology or a related subject with at least 2 years experience in a senior position at an African wildlife institution.

Salary includes a substantial tax-free allowance paid under Britain's overseas aid programme. Basic salary attracts 25% tax-free gratuity.

Benefits include free passages, generous paid leave, children's holiday visit passages and education allowances, outfit allowance, subsidised housing, appointment grant and interest-free car loan.

The terms on which civil and public servants may be released if selected for appointment will be subject to agreement with their present employers.

For full details and application form write quoting MD/297/NA.

Crown Agents

The Crown Agents for Oversea Governments and
Administrations, Recruitment Division,
4 Millbank, London SW1P 3JD.

8534(A)

Forestry Commission, Northern Research Station, Edinburgh

Tree Physiologist

■ Join small team carrying out research on commercially important conifers ■ Design, implement and assess experiments under controlled conditions ■ Maintain field trials in conjunction with other research branches ■ Use wide range of techniques, including the application of plant growth regulators to manipulate the growth of seedlings ■ Assess the potential of micropropagation in commercial forestry ■ Determine the role of environmental factors on flower induction in controlled environments.

☐ Good hons degree or equivalent in botany ☐ At least 2 years' relevant experience essential ☐ Appointment as Higher Scientific Officer (£4100–£5450) ☐ Ref: SA/7/DM.

■ Application forms (for return by 15 December, 1978) from Personnel Branch, Forestry Commission, 231 Corstorphine Road, Edinburgh, EH12 7AT.

8579 (A)

Science Group
CIVIL SERVICE

THE UNIVERSITY OF MANCHESTER
DEPARTMENT OF PSYCHIATRY

A Technician (Grade 4) is required for full-time duties in the Department of Psychiatry, Stopford Building (New Medical School). The successful applicant will work with a small research team studying the effects of drugs on the central nervous system. Duties will include assistance with electrophysiological experiments, photography and general laboratory maintenance. Some knowledge of electronics is an advantage.

The applicant should possess H.N.D. or H.N.C. (or equivalent qualifications) in appropriate subjects and have several years' relevant laboratory experience.

Salary range £2,955 to £3,402 per annum (scale under review).

Consideration will be given for appointment to a lower graded post to candidates possessing an O.N.C. or equivalent qualification.

Applications, together with a curriculum vitae and the names and addresses of two referees, should be sent to Mrs G. Scott, Department of Psychiatry, University Hospital of South Manchester, West Didsbury, Manchester M20 8LR. Closing date for applications, December 8, 1978.

8517(A)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF CHEMISTRY

Applications are invited for a research post to study the use of coherent Raman spectroscopy in combustion. The work will be carried out initially at Southampton University and could lead to a permanent position at Rolls Royce. Applicants should possess a Ph.D. (or in exceptional cases a first degree) and have experience in one or more of the following fields:- laser, combustion, spectroscopy. Applicants with a first degree only will be expected to register for a higher degree. A high degree of experimental ability is desirable. Salary for a research fellowship up to £4,382 per annum. Alternatively for a research studentship, a grant of £1,610 is payable.

Please apply in writing giving curriculum vitae etc., and the names of two referees to Professor I. R. Beattie, Chemistry Department, The University, Highfield, Southampton Hants. SO9 5NH. Please quote ref: MAI/Z/**Na.**

8516(A)

UNIVERSITY OF OXFORD
UNIVERSITY LECTURESHIP IN ELEMENTARY PARTICLE PHYSICS

Applications are invited for the above post. Stipend according to age on the scale (under review) £3,883 to £8,257. The successful candidate may be offered a tutorial fellowship by Balliol College (for either a man or a woman). Details of both appointments may be obtained from Professor K. W. Allen, Department of Nuclear Physics, Keble Road, Oxford OX1 3RH, to whom completed applications (one typed copy) and the names of three referees should be sent by January 31, 1979.

8506(A)

RESEARCH TECHNICIAN for Department of Anatomy to assist group in study of the development of lymphocytes. Extensive tissue culture and use of a variety of immunological techniques are involved. Previous experience and appropriate qualifications essential. Instruction will be given in techniques related to tissue culture and immunology. Salary scale £2,955 to £3,402 per annum. Ref. 401/C/327. Application form from Assistant Secretary, Personnel Office, University of Birmingham, P.O. Box 363, Birmingham B15 2TT.

8529(A)

UNIVERSITY OF NEWCASTLE UPON TYN
DEPARTMENT OF SOIL SCIENCE

Applications are invited for a ne established Lectureship in the Depa ment of Soil Science to take appointment as soon as possible. Ap cants should be qualified to teach the soil chemistry and fertility sect of the subject. Those applicants w special interests *either* in the natı and properties of inorganic/orga soil colloid surfaces *or* in experimen design and data processing may preferred.

Salary will be at an appropri point on the Lecturers' scale: £3, to £7,754 per annum, according to a qualifications and experience. Memb ship of the appropriate Univers superannuation scheme will be quired.

Further particulars may be obtair from the Registrar, The Universi Newcastle upon Tyne NE1 7RU, w whom applications (three copi together with the names and addres of three referees, should be lodged later than January 1, 1979. Ple quote reference N.

8524(A

UNIVERSITY OF WARWIC
SENIOR RESEARCH TECHNICIAN

In the Department of Biologi Sciences, to organise the daily runni of the technical services of the Chlo plast Research Group, and to as with research under the personal dir tion of Professor R. J. Ellis. T research programme is aimed at ravelling molecular interactions tween the nuclear and chloropl genomes. The main techniques u include *in vitro* protein synthesis isolated chloroplasts and wheat ge extracts programmed with mRNA, the analysis of RNA and polypepti by one and two-dimensional polyac lamide gel electrophoresis. Appli tions are invited from suitably qualif persons with several years' experie in biochemistry. Salary on the Te nician Grade 5 scale £3,186 to £3, per annum (under review). Ref. 15/D/78.

RESEARCH TECHNICIAN

The Department of Biologi Sciences has vacancies in the Chlo plast and Developmental Biology search Groups. The work involves isolation and characterisation of p teins and nucleic acids, using stand biochemical techniques. The posts for contract periods of one to th years. Applications are invited fr graduates and others with appropri qualifications in biochemistry and microbiology. Salary either on Technician Grade 2B scale: £2,529 £2,880 per annum *or* Grade 3 £2, to £3,060 per annum (under revie Ref. No: 15/2D/78.

Applications (n/f), for the ab posts should be made by letter quot the appropriate Ref. No. and giv full details to the Personnel Offi University of Warwick, Coventry C 7AL. Closing date for receipt applications is November 30, 1978.

8523(A

SCOTTISH MARINE BIOLOGICAL ASSOCIATIC
Dunstaffnage Marine Researc Laboratory near Oban, Argy
(Partial Readvertisement)

The Association intends to appo a scientist at the H.S.O./S.S.O. le to undertake fundamental research problems related to mariculture. Ap cants should have at least two ye postdoctoral experience in the field fish physiology/behaviour/embryolog

For further particulars and an ap cation form please apply in writing the Administrative Officer, Scott Marine Biological Association, P Box 3, Oban, Argyll PA34 4A Closing date for applications Dec ber 21, 1978.

8578(A

POSTDOCTORAL POSITION BIOCHEMISTRY

To study interferon-carbohydrate iteraction. Background in tissue cul- ire essential, experience with viral ssays desirable. The position is available immediately, the salary is $12,000 er annum, or more depending on revious experience. Send inquiries, icluding curriculum vitae and names f three references, to:

Dr Helmut Ankel
Department of Biochemistry
Medical College of Wisconsin
8701 Watertown Plank Road
P.O. Box 26509
Milwaukee, Wisconsin 53226

8560(A)

UNIVERSITY OF CALIFORNIA
SANTA CRUZ
BOARD OF STUDIES IN ASTRONOMY AND ASTROPHYSICS AND STEVENSON COLLEGE

Applications are invited for the position of:

ASSISTANT PROFESSOR OF ASTROPHYSICS

effective September 1, 1979

Appointee will have graduate and ıdergraduate teaching and advising sponsibilities, will be expected to ount a research program in the oard, and to participate on the aca- :mic and intellectual life of the ollege. Position is in regular tenure- ack series; initial appointment is for vo years. Applicants must hold Ph.D. expect to receive it by September 1979.

Persons active in theoretical astro- ıysics with interests in a broad ıriety of problems, and who would elcome the opportunity of interaction ith astrophysicists, physicists and ick Observatory astronomers, and ho have a strong commitment to aching are preferred. Applications om non-optical, non-IR observational stronomers who have a strong theor- ical component to their research iterests will also be considered.

Salary, $15,100 to $15,700, nine onth basis. Applicant should send ırriculum vitae, list of publications, atement of research interests and aching experience, together with ımes of at least three references who ıve been asked to submit letters of commendations directly to us. All ıpporting materials should be sent, med to arrive not later than January 5, 1979, to:

Dr Robert P. Kraft, Chair
Board of Studies in Astronomy
and Astrophysics
Lick Observatory
University of California
Santa Cruz, CA 95064
(An Affirmation Action/Equal
Opportunity Employer)

8557(A)

'HE UNIVERSITY OF HULL
SENIOR LECTURER/ LECTURER IN APPLIED PHYSICS

Applications are invited for the post Senior Lecturer/Lecturer in the epartment of Applied Physics for ork on satellite laser ranging, tenable om a date to be arranged.

Salary scale: £3,883 to £9,263 per ınum (under review) plus superannu- ion benefits.

Applications (six copies) giving tails of age, qualifications and ex- rience, together with the names of ree referees, should be sent by ecember 4, 1978 to the Registrar, ıe University of Hull, Hull HU6 :X from whom further particulars ay be obtained. 8526(A)

Applications are invited for a

FACULTY POSITION IN ZOOLOGY

beginning September 1, 1979. Candidates are sought at, but not restricted to, the Assistant Professor level. Applicants should have a research background in structural–functional relationships at analytical ultrastructural, physiological, and biochemical levels, with emphasis on such areas as cell–cell interactions, cell motility, cell surface phenomena and/or membranes. Applicants should have sufficient breadth to be able to contribute to undergraduate teaching at the organismal level, as well as in their specialty.

Send application letter, statement of research plans, curriculum vitae, including teaching and funding experience, sample reprints, and names of five referees to: Dr A. G. Jacobson, Department of Zoology, University of Texas at Austin, Austin, Texas 78712.

Application deadline: December 15, 1978.

An equal opportunity/affirmative action employer. 8497(A)

M.I.T.
Postdoctoral and Predoctoral Research Positions

Several positions are available for research in genetic toxicology with emphasis on mechanisms of chemical mutation of human cells. Strong background in chemistry is desired of predoctoral researchers, and molecular biology of postdoctoral researchers. Please send resumes to Professor William G. Thilly, Room E18-666, M.I.T., Cambridge, MA 02139. M.I.T. is an equal opportunity employer.

8392(A)

SIMON FRASER UNIVERSITY
BRITISH COLUMBIA, CANADA
DEPARTMENT OF BIOLOGICAL SCIENCES
ENTOMOLOGIST

The Department of Biological Sciences invites applications for a tenure track position in entomology. The successful applicant will be expected to develop a strong research programme and to participate in the teaching programme at both the undergraduate and graduate level including courses in the Master of Pest Management Programme. Candidate must have a Ph.D. degree in entomology or related subjects; preference may be given to individuals with teaching and research experience in agricultural pest management. The position is available, subject to budgetary constraints, from September 1, 1979. The initial appointment is for a period of two years, renewable. Salary will depend on experience; the base salary for the Assistant Professor rank currently is $18,604 per annum.

Applications, including an up-to-date curriculum vitae, reprints of published research, and the names of three referees should be sent to: Dr M. Mackauer, Chairman, Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada, V5A 1S6. Deadline for receipt of applications is January 31, 1979 or when position filled. 8390(A)

OXFORD UNIVERSITY
UNIVERSITY LECTURESHIP IN EXPERIMENTAL PHYSICS

Applications are invited for a University Lecturership in Experimental Physics in the Clarendon Laboratory (which may be held in association with an Official Tutorial Fellowship at Worcester College). Preference will be given to candidates whose research is in the general field of atomic and radiation physics.

Further particulars may be obtained from Professor E. W. J. Mitchell, The Clarendon Laboratory, Parks Road, Oxford OX1 3PU, to whom applications should be sent by December 15, 1978. 8522(A)

Solomon Islands
Senior Forestry Officer
Up to £9,200 plus allowances

Candidates should have a degree or diploma in Forestry with relevant management and work study experience, preferably in the tropics.

The work will involve general forestry duties including surveys, co-ordinating costs and records at field stations, and training local staff.

Salary includes a substantial tax-free allowance paid under Britain's overseas aid programme. Basic salary attracts 25% tax-free gratuity.

Benefits include free passages, generous paid leave, children's holiday visit passages and education allowances, outfit allowance, subsidised housing, appointment grant and interest-free car loan.

The terms on which civil and public servants may be released if selected for appointment will be subject to agreement with their present employers.

For full details and application form write quoting MD/1036/NA.

Crown Agents

The Crown Agents for Oversea Governments and Administrations, Recruitment Division, 4 Millbank, London SW1P 3JD.

8501(A)

Research Biochemist

Roche Products Limited is part of a major international pharmaceutical Company based in Switzerland and is itself one of the leaders of the industry in the United Kingdom.

We currently have a vacancy for a Research Biochemist, male or female, in our Biochemistry Department, part of a multi-disciplinary Research Function based at Welwyn Garden City. The work involves investigating novel antithrombotic and antihypertensive agents.

Applications are invited from those with B.Sc. (or equivalent) in Biochemistry/Pharmacology and several years' research experience in the cardiovascular area. A knowledge of haemotology and/or lipid biochemistry is desirable.

Our Biochemistry Department is located in modern laboratories where the working conditions and facilities are excellent. Conditions of Service include a Christmas Award, 4 weeks' annual holiday, free life assurance and BUPA membership, contributory pension fund and subsidised restaurant.

If you would like to apply for this post, please write briefly or telephone for an application form (Welwyn Garden 28128) to Mrs Clewley, Research Personnel Department, quoting reference RB30. Roche Products Limited, P.O. Box 8, Welwyn Garden City, Herts AL7 3AY.

8574(A)

ROCHE

Plant Pathologists/ Fungal Physiologist

Shell Research Limited is seeking two plant pathologists, one of whom could be a Fungal Physiologist, to join the reseach staff of Shell Biosciences Laboratory at Sittingbourne, Kent.

One of the posts is in the Fungicides Section of the Biological Evaluation Division where you should have a good background in plant pathology, probably to Ph.D. level, together with an understanding of the principles of crop protection. Whilst the work will entail the evaluation of new fungicides at several stages, it is anticipated that you will be capable of assuming responsibility for a major share of the field development programmes in the UK and overseas. An ability to present good written reports is essential.

The other post (Fungal Physiologist) is within the Plant group of our Physiology and Biological Chemistry Division where you will be responsible for initiating and conducting research projects on a biochemical/physiological level, in areas of plant pathology, mycology or fungicide mode of action. You should have a good first degree and research experience in plant pathology or mycology.

For either post you should be aged between 24-29 years. We offer a competitive salary and assistance will be given with relocation expenses where appropriate. You will be able to join our pension fund, enjoy flexible working hours and work in well-equipped laboratories which are situated on a 450 acre farmland site. There are also excellent facilities for sports and social activities. Please write giving all the relevant details about yourself and your career or telephone for an application form to:

Shell Research Limited, Recruitment Division PNEL/11 (N1), Shell Centre, London SE1 7NA. Telephone: 01-934 2948.

Shell

8567 (A)

UNIVERSITY OF SINGAPORE

MATHEMATICS

Applications are invited for teaching appointments, ranging from Lectureships to Professorships, in the Department of Mathematics. Candidates with special qualifications in numerical analysis and/or operations research and able to teach computer science are preferred. Gross monthly emoluments in the range from S$1,445 to S$5,050 approx., point of entry depending on the candidate's qualifications and experience and level of appointment offered. In addition, the University pays a 13th month annual allowance of one month's salary in December of each year. Leave, subsidised housing, medical and provident fund benefits are available. Candidates should write to: **The Registrar, University of Singapore, Kent Ridge, Singapore 5,** giving curriculum vitae (bio-data) with full personal particulars and also the names and addresses of three referees.

8500(A)

UNIVERSITY OF LEICESTER

DEPARTMENT OF MICROBIOLOGY

LECTURESHIP IN VIROLOGY

Applications are invited for the post of non-clinical LECTURER in Virology in the Department of Microbiology (Head of Department, Professor P. H. A. Sneath, M.D.). The Department is responsible for both clinical and pre-clinical teaching in the Medical School and also provides courses for honours science students. The successful applicant will be expected to take part in teaching both medical and science students. Interest in genetics, immunology or electron microscopy will be welcomed. There are excellent research facilities.

Initial salary dependent on qualifications and experience, on the scale £3,883 to £7,754 (under review) with superannuation benefits.

Further particulars from the Registrar, to whom applications should be sent by December 15, 1978, quoting reference LVN. 8499(A)

ROTHAMSTED EXPERIMENTAL STATIO

HARPENDEN, HERTS. AL5 2JQ

SCIENTIFIC OFFICER/ HIGHER SCIENTIFIC OFFICER

required for temporary one-y
appointment from January 1979 in t
Soils and Plant Nutrition Departme
to join a team investigating the use
liquid nitrogen fertilisers and nitrifi
tion inhibitors, in the field and in
laboratory. First or Upper Sec
Class degree in soil science, chemis
or allied discipline required. Exp
ence of agriculture or field exp
mentation an advantage; driving lice
desirable. Appointment in grade
Scientific Officer (£2,839 to £4,415)
Higher Scientific Officer (£4,101
£5,448). At least two years' appropri
research experience is required
appointment in the H.S.O. grade. N
contributory superannuation.

Apply in writing to the Secret
giving names and addresses of
referees and quoting Ref. 379
November 30, 1978. Further particu
on request. 8503(A

UNIVERSITY OF DUNDEE

The University has established a new post of

DIRECTOR OF ANIMAL SERVICES

and applications for it are invited from appropriately qualified and experienced candidates of either sex

The successful candidate will be responsible to the Dean of the Faculty of Medicine and Dentistry for all aspects of the running of the Animal Units in the Faculty's premises at Ninewells Hospital and Medical School, Dundee and in the main Univer-ity precinct near the city centre Research facilities and office accommodation for the Director and a secretary (to be appointed) are available at Ninewells.

Salary scale £7,506 to £9,263 (under review) with appropriate initial placing. Superannuation under U.S.S. (or continuation of F.S.S U). Grant towards removal expenses to Dundee.

Further particulars are available from The Secretary, The Univer-ity, Dundee DD1 4HN with whom applications (six copies) containing details of qualifications and experi-ence and the names of three referees should be lodged by December 15. Please quote reference EST/66/78J

8542(A)

THREE POSTDOCTORAL POSITIONS

 available immediately in the llowing areas:

(1) Synthesis and utilisation of cleotide analogues in investigation the mechanism of action of the mbrane ATPase responsible for TP-driven transport and ATP syn-esis in bacteria and mitochondria. r Alan Senior)

(2) Divalent cation involvement in e membrane ATPase of bacteria and itochondria; role of the very tightly-und nucleotides in this enzyme r Alan Senior).

(3) Transport and metabolism of gars and amino acids in mammary mour cells. Influence of hormones, d studies of hormone receptor ulation (Dr Russell Hilf).

Salaries $12,000 to $14,000, plus 8 r cent benefits Send curriculum ae to:

Dr Alan E. Senior or
Dr Russell Hilf
Department of Biochemistry
Box 607
University of Rochester
Medical Center
Rochester, New York 14642

An Equal Opportunity/Affirmative Action Employer

8563(A)

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(University of London)

INCHES FARM FIELD STATION, ST ALBANS

JUNIOR MEDICAL LABORATORY SCIENTIFIC OFFICER "B"

required for a permanent appoint-ent to assist in research in the epartment of Medical Helminthology andidates should have passes in C E "A" level Biology or Zoology d Chemistry, and "O" level passes English and Mathematics; or alter-tively an O.N.C in Biological iences, and have a knowledge of or perience in histology Salary, de-nding on age and qualifications, es to a maximum of £3,161

Written applications, giving full reer and education details and nam-g two referees, should be sent to the cretary (A1), Winches Farm Field ation, 395 Hatfield Road, St Albans, erts. 8570(A)

CSIRO

AUSTRALIA

Research Scientist/ Postdoctoral Research Fellow

(2 Positions)

Division of Atmospheric Physics Aspendale, Victoria

CSIRO has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: Dynamical Meteorology/Dynamical Oceanography

General: The Division of Atmospheric Physics is engaged in research in three broad areas: Dynamical Meteorology, Radiation and Climate and Atmospheric Chemistry. The Dynamical Meteorology programme comprises individual research projects on a variety of topics including large scale atmospheric circulations, the dynamics of stratified and rotating fluids, convection, mixing and entrainment, the atmospheric boundary layer and air/surface interaction. The programme also includes studies on the effects of regional and topographic factors on weather and climate and the circulations in the waters surrounding Australia.

Wherever possible the approach to these topics combines analytical, numerical and laboratory modelling techniques, and field experiments. The Division has an active and well equipped geophysical modelling laboratory and, in addition to local computers, has access to the CSIRO's CYBER network. Interaction with the Australian Government Bureau of Meteorology, the Australian Numerical Meteorology Research Centre and the CSIRO Divisions of Cloud Physics and of Fisheries and Oceanography is encouraged.

Duties: To conduct appropriate theoretical and experimental research on topics concerning the large scale dynamics of the atmosphere or dynamical oceanography relevant to the southern hemisphere weather and climate.

Qualifications: Applicants should have a strong background in geophysical fluid dynamics and a Ph.D. or equivalent qualification, and postdoctoral experience demonstrating a high level of ability in self-motivated and original research.

Salary: Research Scientist or Senior Research Scientist: $A14,829—$A21,543 p.a. Outstandingly qualified scientists should not be deterred from applying as appointment at a higher level would be considered.

Tenure: One appointment will be of indefinite tenure. For the other either an indefinite or a fixed term appointment may be negotiated. Both carry superannuation benefits.

Applications **in duplicate**, stating **full** personal and professional details, the names and addresses of at least two professional referees, and **quoting reference number 420/352** should reach:—The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, London WC2R 3EH by 22nd December 1978.

Applications in U.S.A. and Canada should be sent to:—The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A. 8552(A)

AN FORAS TALUNTAIS

GRADUATE IN CHEMISTRY, BIOCHEMISTRY, OR FOOD SCIENCE

required for research on quality aspects of fresh and processed horticultural crops at Kinsealy Research Centre, Malahide Road, Dublin 5. **Essential:** Honours University degree or equivalent majoring in Chemistry, Biochemistry or Food Science. **Desirable:** Suitable research experience.

Appointment up to and including Senior Research Officer level (salary scale £6,459 to £8,465 p.a.) depending on qualifications and experience Non-contributory pension scheme with provision for Widows' and Orphans' Pensions.

Application forms and further particulars from the Personnel Department, An Foras Taluntais, Headquarters, 19 Sandymount Ave., Dublin 4, Tel: 01-688188. Latest date for receipt of completed forms: Friday. December 15, 1978. 8518(A)

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF MICROBIOLOGY

Applications are invited from men and women for the following post financed by S R.C, tenable for 3 years from a date as soon as possible to be arranged· Postdoctoral research assistant to work on the mechanism of methanol assimilation in yeasts, involving isotopic work with whole cells and purification and study of enzymes Applicants should possess, or expect soon to possess a Ph D in microbiology or biochemistry. Salary up to £4,382 a year on Range IA. Particulars from Professor J. R Quayle in the above Department to whom applications, quoting two referees, should be sent by December 11, 1978. Quote ref: R226/G. 8551(A)

UNIVERSITY OF LONDON KING'S COLLEGE

DEPARTMENT OF PLANT SCIENCES

GRADUATE RESEARCH ASSISTANT

A Biochemistry/Biology graduate is required immediately, until September 30, 1979, in the Department of Plant Sciences, to work on a project involving the isolation and purification of glycine decarboxylase from C_3 plant mitochondrian under the supervision of Dr A. L. Moore and Professor D O. Hall.

Please send a curriculum vitae and the names of two referees to Dr A. L. Moore, Department of Plant Sciences, University of London, 68 Half Moon Lane, London SE24 9JF quoting reference 191649/N. 8533(A)

BUSH BOAKE ALLEN LIMITED, a division of Albright and Wilson Ltd., have a vacancy in the Process Technology Department of the Flavour Laboratories at Walthamstow, London E17, for a

SENIOR PROCESS TECHNOLOGIST

The principle duties of the successful candidate will be to follow lines of research in connection with onion flavours. In particular he/she will study the relationship between varieties and stages of maturity of onions with their yields of flavour precursors.

Applications are invited from candidates aged 24+ with a good academic knowledge probably at postgraduate level, experience with natural products and their chemistry and handling is essential. A knowledge of, or experience with H.P.L.C. is desirable.

An attractive salary will be offered in addition to a range of employee benefits which include a non-contributory pension scheme.

Applications in writing to:

R. J. Saunders,
Recruitment and Training Manager,
BUSH BOAKE ALLEN LTD.,
Blackhorse Lane,
Walthamstow E17
Tel: 01-531 4211

stating age, qualifications and experience.

8571(A)

Bush Boake Allen

Applications are invited for the position of:

PROJECT LEADER

in a

DRUG METABOLISM AND PHARMACOKINETICS GROUP

with a leading Pharmaceutical Company in Paris

The applicant should have a particular knowledge of h.p.l.c. methods of analysis and a Ph.D. degree in a relevant discipline. Send applications and curriculum vitae to

NATURE
Box No. 8576(A)
3 Dyers Buildings, London EC1N 2NR

All replies will be treated in the strictest confidence

8576(A)

POSTDOCTORAL POSITION

available in laser light scattering laboratory. Background in polymer solution chemistry or physical biochemistry required Contact Professor F. E. Karasz, Department of Polymer Science and Engineering, or Professor N. C Ford, Department of Physics, University of Massachusetts, Amherst, 01003 An Equal Opportunity/Affirmative Action Employer 8466(A)

STRUCTURAL BIOLOGIST

The Department of Biological Sciences, Carnegie-Mellon University, invites applications from individuals whose research interests include the use of biochemical and biophysical techniques to study biological structures. The candidate should have postdoctoral experience. The position is at the assistant or associate professor level. Responsibilities include teaching at the undergraduate and graduate level, and carrying out an active research program The applicant should forward a curriculum vitae, three or more letters of reference, and a brief résumé of research plans to Search Committee (B), Department of Biological Sciences, Carnegie-Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213, U.S.A.

Carnegie-Mellon University is an Equal Opportunity/Affirmative Action Employer. Please respond by December 15, 1978. 8446(A)

MEDICAL RESEARCH COUNCIL

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

IMMUNOLOGIST

A young postdoctoral immunologist is required by the LABORATORY FOR LEPROSY AND MYCOBACTERIAL RESEARCH to investigate immunity of mycobacterial infections. It is proposed that the person appointed will study particularly the immunological control of the growth of mycobacteria in tissue culture and in animals. The appointment will be for up to three years and will be in the salary range £4,655 to £6,080 per annum according to age and qualifications, plus £450 per annum London Allowance Superannuation provision

Applications, giving details of qualifications, experience and the names of two professional referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA before December 31, 1978, quoting reference L 1.

8504(A)

McGILL UNIVERSITY

FACULTY OF MEDICINE

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

Faculty positions open in

Developmental Pharmacology
Drug Biotransformation
Antitumor Pharmacology

Applications are invited from persons with an M.D. or Ph.D. degree who have research and teaching interests in the above fields. Salaries and professional rank will be commensurate with qualifications and experience. Curriculum vitae, bibliography, summary of research interests and names of three referees should be sent to Dr R I. Ogilvie, Chairman, Department of Pharmacology and Therapeutics, McGill University, 3655 Drummond Street, Montreal, Quebec, Canada H3G 1Y6.

8550(A)

GRADUATE required to assist in cell biological work directed to cancer research and in particular to the study of diseases with defects of DNA metabolism Experience in cell biology, cytogenetics or other relevant fields an advantage. Applications to the Administrative Assistant, Paediatric Research Unit, The Prince Philip Research Laboratories, Guy's Hospital Medical School, London Bridge SE1 9RT. 8546(A)

OKLAHOMA MEDICA
RESEARCH FOUNDATI

Applications are invited fro

BIOCHEMISTS

with Ph.D. for a RESEARCH A
CIATE position available a
December 1, 1978 for up to
years for structural studies
apolipoproteins. Experience in p
separation and characterisation
able and in trace metals ha
Starting salary range $12,00
$13,500 depending on backgr
Excellent benefits and working
ditions Send curriculum vitae, de
tion of research experience and
niques, two letters of reference
copy of college transcripts as so
possible to Dr Diana Lee, Labor
of Lipid and Lipoprotein St
Oklahoma Medical Research Fo
tion, 825 N.E. 13th Street, Okla
City, Okla. 73104, U.S.A.

Equal Opportunity/Affirmativ
Action Employer.

8556

UNIVERSITY OF KEN
AT CANTERBURY

BIOLOGICAL LABORATOR

POSTDOCTORAL
RESEARCH FELLOW

Applications are invited for
position of Postdoctoral Res
Fellow, supported by the Well
Trust, in a group led by Drs K.
and C. L Pogson. The research p
concerns the drug sensitivity of n
tubular systems from a rang
eucaryotic organisms, adequate
nical support will be available.
expected that the successful app
will be able to start early in
Previous experience of protein
istry or enzymology will be an a
tage. The appointment, for three
will be on the scale £3,883 to £

Application forms and further d
may be obtained from the
Assistant Registrar, Faculty of N
Sciences, Chemical Laboratory,
University, Canterbury, Kent
7NH Closing date for applicati
December 22, 1978. Please quot
A42/78 8561

TECHNICIAN (Grade 5) requir
Department of Microbiology, U
sity of Reading Main duties in
supervision of laboratory servic
section dealing with teaching an
search in Microbial Physiology
Biochemistry; equipment for tea
and research, chemical stores.
according to qualifications and e
ence in range £3,186 to £3,72
(under review). Apply, stating
fications and experience and
the names of two referees, q
Ref. T.N 74A, to Assistant
(Personnel), University of Re
Whiteknights, Reading RG6 2A
8549

BUMPUS PROFESSORS

Brown University invites applic
from qualified candidates in Vert
Ecology for appointment as
Hermon Carey Bumpus Profes
Biology. Applicants should hav
established program in vert
ecology, a specific interest in
tionary biology, and be qualifi
relate to such areas as beha
genetics, morphology, and physi
A candidate must have the Ph.
gree or its equivalent. In additi
carrying out an original research
gram s/he will participate in the
tual development of a graduate
gram in evolutionary biology.
ing responsibilities include vert
ecology plus another course i
candidate's area of expertise
cants should send curriculum
bibliography, a brief description
search and teaching, and name
addresses of three references by
ary 31, 1979 to Richard J. Goss,
of Biological Sciences, Divisi
Biology and Medicine, Brown
versity, Providence, Rhode
02912 Brown University is an
Opportunity/Affirmative Action
ployer. 856

UNIVERSITY OF SURREY
DEPARTMENT OF HUMAN BIOLOGY AND HEALTH
TECHNICIAN GRADE 6

Required as deputy to the Chief Technician and to be responsible under him for the operation of the teaching laboratories of the Department and the day to day supervision of a technical staff of six. The laboratories teach physiology, anatomy and related subjects in human biology to honours degree level.

Applicants should possess relevant H.N.C., Advanced City & Guilds Certificate, or similar qualifications, and particularly have considerable experience of this type of laboratory work, with high technological awareness and practical skill. Experience of supervising technicians and an ability to communicate with other groups of staff is also desirable.

The Department is in a new building in attractive surroundings, adjacent to an historic town with excellent recreational facilities on the campus and in the surrounding area.

Further particulars and an application form may be obtained from the Staff Officer. University of Surrey, Guildford, Surrey GU2 5XH or telephone Guildford 71281, ext. 452.

8573(A)

POSITION AVAILABLE

For young Ph.D. in biochemistry interested in working on the comparative protein chemistry of the major structural proteins of higher cells. Experience in sequence analysis or protein chemistry would be helpful. The group is bilingual.

Salary according to experience (not less than DM1,900=~$1,000 or £512 per month tax free).

Application to Klaus Weber, Max Planck Institute for Biophysical Chemistry, D-34 Göttingen, West Germany.

8566(A)

University of Oxford
NUFFIELD INSTITUTE FOR MEDICAL RESEARCH
POSTDOCTORAL RESEARCH ASSISTANT

To join a group working on growth and development of the fetus. The area of investigation is the control of lung maturation and will concentrate on the ontogeny of lung specific proteins. Experience in protein isolation and radioimmunoassay techniques would be advantageous.

The post is M.R.C. supported for five years (January 1, 1979 to December 31, 1983). Salary on Research Scale 1A (£3,883 to £6,555).

Applications, with curriculum vitae and the names of two referees, should be sent to:

The Administrator
Nuffield Institute for
Medical Research
Headley Way
Headington
OXFORD
OX3 9DS.

8539(A)

UNIVERSITY OF GLASGOW
CHAIR OF VETERINARY PARASITOLOGY

Applications are invited for a new Chair of Veterinary Parasitology. A veterinary qualification is desirable.

Further particulars may be had from the Secretary of the University Court (Room 18), University of Glasgow, Glasgow G12 8QQ, with whom applications (15 copies; in the case of overseas applicants, one copy), giving the names and addresses of three referees, should be lodged on or before January 15, 1979.

In reply please quote Ref. No. 311M.

8555(A)

BIOLOGIST/PHYSIOLOGIST

required by the Biology Section of the Medical Research Council Cyclotron Unit. The successful applicant will join a group investigating responses of normal tissues and tumours in animals relevant to the treatment of cancer. The post will primarily be concerned with damage to normal tissues, particularly the central nervous system. Studies will include effects of both neutrons and X-rays and certain adjuvant treatments used in radiotherapy. The applicant must have a First or Upper Second Class degree in an appropriate subject and at least three years' relevant postgraduate research experience. Salary, according to age and experience, will be in the range £5,081 to £6,530 (inclusive of pay supplements and London Weighting), plus six weeks annual leave. Please write, giving qualifications and curriculum vitae, to: Miss A. Pires, M.R.C. Cyclotron Unit, Hammersmith Hospital, Ducane Road, London W12 0HS. Tel: 01-743 **4594,** Ext. 103.

8498(A)

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOLOGICAL SCIENCES

Applications are invited for a

RESEARCH ASSISTANT

to work in the above Department under the supervision of Dr R. A. Herbert. The post is financed by N.E.R.C. and will be available for up to three years. The investigation is on the interactions of sulphate reducing bacteria and photosynthetic bacteria in marine sediments. Applicants should have an Honours degree in Microbiology.

Salary scale: £3,384 to £3,883 per annum.

Applications, in writing, containing the names of two referees should be sent by December 14, 1978 to The Secretary. The University, Dundee DD1 **4HN. Please quote** Reference EST/65/78J.

8530(A)

UNIVERSITY OF DUNDEE
DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

There is an immediate vacancy in the above Department, based at Ninewells Hospital and Medical School, Dundee, **for a**

RESEARCH TECHNICIAN

to join a group engaged in a study of 'oncofetal proteins.

Experience of protein purification techniques and radioimmunoassay is desirable though not essential.

An applicant with an appropriate H.N.C. or equivalent qualification and at least eight years relevant experience would be eligible for appointment at a salary of up to £3,720 per annum but an appointment at a lower grade could be offered to a less well-qualified and experienced applicant.

Applications, giving details of qualifications and experience and the names of two referees should be sent to The Secretary, The University, Dundee DD1 **4HN** by **December** 7, 1978. Please quote Ref: EST/170/78J.

8531(A)

LABORATORY Scientific Officer required from March 1, 1979 for work on a three-year research project, supported by the Medical Research Council, examining the effect of steroids on experimental and human immune complex disease. Techniques used will include basic immunochemical methods, analytical ultracentrifugation, tests of macrophage function and electron microscopy. Salary for qualified Laboratory Scientific Officer not less than £3,261 plus £354 London Weighting. Apply in writing, stating age and giving details of qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref. R.U.I.

8545(A)

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF BOTANY

Position immediately available as

EXPERIMENTAL OFFICER

in the A.R.C. Group working on the fusion of plant protoplasts. Appointment for one year in the first instance. Applicants should possess a degree, preferably in the biological sciences. Salary scale (£3,384, £3,634, £3,883).

Applications to Professor E. C. Cocking, Department of Botany, University of Nottingham, University Park, Nottingham NG7 2RD.

8525(A)

UNIVERSITY OF BRISTOL
MATHEMATICAL STATISTICS

Applications are invited
from probablists and statisticians
for the post of

LECTURER IN STATISTICS

in the School of Mathematics.

The person appointed will have the responsibility for teaching undergraduates in a combined mathematics and statistics degree programme and for supervising the research of postgraduate students in Probability and Statistics. Personal research interests should lie in the same fields.

The post is tenable from August 1, 1979 **and the** initial salary will be within the range £3,883 to £6,317 per annum on the current salary scale for Lecturers.

Further particulars may be obtained from the Secretary, University Senate House, Tyndall Avenue, Bristol BS8 1TH, **to whom** applications should be sent by December 15, 1978 (please quote reference EB).

8502(A)

APPTS. VACANT CONT'D.

QUALIFIED Laboratory Scientific Officer required in Physiology Department, to assist with teaching laboratory duties and research. Salary not less than £3,261 on scale rising to £4,680, plus £354 London Weighting. Apply in writing to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref. P.Y.I.

8544(A)

POSITIONS WANTED

GEOLOGIST, Ph.D., Jan 1979. Spec. sedimentology, inorg. geochem. Long exp. teaching, uranium prosp., X-ray diffract., X-ray fluores. A.A.S., D.T.A., fluorimetry. Shipboard and overseas exp. Participation in inter. sci. proj. Organisation talent. Publ. Perfect English and German. 39 years, single. Europe, Australia. Box No. 8564. Nature, 3 Dyers Buildings, Holborn, London EC1N 2NR.

8564(B)

AGRONOMIST (B.A., M.A.) (Cantab). SPECIALIST RESEARCH/ADVISORY AGRONOMIST IN TEA seeks appointmen with any Research or Advisory Organisation, or Tea Company on Consultancy or Short Term Contract Basis. In my 50's and offer over 22 years' Research and Advisory experience in Ceylon, East and West Africa, Madagascar and Morocco. BILINGUAL. Fluent French. Also 2½ years SUGAR CANE Research, NATAL. Please reply to Nature, Box No. 8553(A) 3 Dyers Buildings, London EC1N 2NR.

8553(B)

SYMPOSIA

CHEMISTRY OF THE PYRETHROIDS
ST CATHERINE'S COLLEGE, OXFORD, JULY 16–19, 1979

The Fine Chemicals and Medicinals Group of the Chemical Society and **the Pesticide Group of the Society** of Chemical Industry are arranging a **symposium on 'Recent Developments** in the Chemistry of Pyrethroids' at **St Catherine's College, Oxford from** July 16–19, 1979.

The symposium will cover aspects of the chemistry of both natural and **synthetic compounds, eg novel** synthesis and synthetic methodology, novel **structures, process chemistry, photochemistry,** biosynthesis, and stereochemistry. **The organising committee has therefore** approached leading academic and **industrial workers for invited papers** to achieve a balanced and attractive **programme.**

Principal participants include—

Professor J. E. Casida, University of California
Professor L. Crombie, University of Nottingham
Dr M. Elliott, Rothamsted Experimental Station
Professor K. Kondo, Sagami Research Centre
Dr J. Martel, Roussel Uclaf
Professor R. A. Raphael, University of Cambridge
Professor I. Yamamoto, Tokyo University of Agriculture

and invited papers will also be contributed by—

Bayer; Ciba-Geigy; FMC; ICI; Roussel Uclaf; Shell;
Sumitomo; Zoecon.

Intending participants who wish to receive the final programme and details of **accommodation and registration** costs should write to:

Dr P. Doyle
ICI Plant Protection Division
Jealott's Hill Research Station
Bracknell, Berkshire RG12 6EY
England

Anyone wishing to contribute a short paper should submit by December 31, 1978, a title, names of authors (speaker underlined) and a summary of 200–250 words to assist the organising committee to select papers consistent with the aims and scope of the symposium.

8538(M)

STUDENTSHIPS

UNIVERSITY OF KENT AT CANTERBURY
BIOLOGICAL LABORATORY
RESEARCH STUDENTSHIP

Applications are invited for a C.A.S.E. studentship, funded by the Science Research Council and May & Baker Ltd., and leading to a Ph.D. degree. The research topic is concerned with the role of the microtubular system in liver metabolism. **The starting date will be January 1. 1979 as as soon as possible thereafter.**

Application forms and further particulars may be obtained from the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH. The closing date for applications is December 8, 1978. Please quote ref: PG15/78.

8562(F)

FELLOWSHIPS

POSTDOCTORAL FELLOWSHIP

Available from January 1, 1979 for a Ph.D. graduate in biochemistry, virology or related area interested in studying the structure and replication of mammalian picornaviruses. Applicants must have received the Ph.D. degree no earlier than April 1, 1978. Salary is $11,600 for the first year.

Send curriculum vitae and references to Dr D. G. Scraba, Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7 Canada.

8388(E)

FELLOWSHIPS CONT'D

EMBO
European
Molecular Biology Organization
LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY SPRING 1979 AWARDS

Next deadline: February 20, 1979

The long term fellowships will usually be for a period of up to one year but applications for a renewal for a second year are considered. To be eligible a candidate must hold a doctors degree. Preference will be given to European and Israeli applicants wishing to work within Europe or Israel. EMBO long term fellowships are **not,** however, awarded for exchanges between laboratories within any one country. Applications for fellowships to be held outside Europe and Israel are considered but they have a lower priority, as do applications from non-European scientists wishing to work in Europe or Israel.

The deadline for applications for the 60 fellowships to be awarded in April '79 is February 20, 1979. Because the selection procedure involves an interview this deadline should be respected.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F.R. Germany. 8520(E)

LA TROBE UNIVERSITY
Melbourne, Australia
SCHOOL OF PHYSICAL SCIENCES
DIVISION OF THEORETICAL AND SPACE PHYSICS

RESEARCH FELLOWSHIP

A Research Fellowship is available in the Division of Theoretical and Space Physics for work on theoretical problems in wave-particle interactions in the earth's magnetosphere. The Research Fellow will work with Professor K. D. Cole from whom further details can be obtained.

The successful candidate may be appointed for a period of up to two years and would be expected to take up his position as early as possible in 1979.

Salary: $A15,860 per annum. Some travel support to take up the appointment is available.

Further information and application forms available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications close with the Staff Officer by **January 31, 1979.**

8510(E)

QUEEN MARY COLLEGE
University of London
CHEMISTRY DEPARTMENT
Applications are invited for a

POSTDOCTORAL RESEARCH FELLOWSHIP

for work with Professor R. Bonnett (in collaboration with St Mary's Hospital Medical School) on the development of tetrapyrrole photosensitisers. The project would suit an organic chemist with experience in heterocyclic synthesis, but an interest in biochemistry and photochemistry would be an advantage. Starting date January 1, 1979 or as soon as possible thereafter. Salary in range £4,359 to £4,858 p.a. (under review, including London Allowance). Please apply by letter giving age, qualifications, experience and names of 2 referees to The Registrar, (N) Queen Mary College, Mile End Road, London E1 4NS.

8509(E)

COURSES

A COURSE ON CLINICAL IMMUNOLOGY

is to be held in Edinburgh, April 2–6, 1979
in Room 8, William Robertson Building, George Square

The object of this 5-day course is to provide a synopsis of modern basic immunology as applied to clinical medicine. The course is comprised of the following topics and speakers:

Introduction to immunology	1.	Dr W. J. Irvine, Royal Infirmary, Edinburgh.
The macrophage and immunity	2.	Dr D. M. Weir, University of Edinburgh, Edinburgh.
Allergy	3.	Dr W. E. Parish, Unilever Research, Bedford.
Renal diseases	4 & 5.	Professor D. K. Peters, Royal Postgraduate Medical School, Hammersmith Hospital, London.
HLA-system and disease	6.	Dr J. R. Batchelor, Queen Victoria Hospital, East Grinstead.
The immunology of viral infections	7.	Dr Pamela Ewan, Royal Postgraduate Medical School, Hammersmith Hospital, London.
Clinical Case—the immunology of non-viral infections	8.	Dr C. M. Feek, Royal Infirmary, Edinburgh.
Endocrine diseases	9 & 10.	Dr W. J. Irvine, Royal Infirmary, Edinburgh.
Immunodeficiency diseases	11.	Dr A. D. B. Webster, Northwick Park Hospital, Harrow.
Haematology	12.	Dr A. C. Parker, Royal Infirmary, Edinburgh.
Chest diseases	13.	Professor J. Pepys, Cardiothoracic Institute, London.
Liver diseases	14.	Dr R. Williams, King's College Hospital Medical School, London.
Dermatology	15.	Dr R. St C. Barnetson, Royal Infirmary, Edinburgh.
Neurology	16.	Dr H. Valdimarsson, St Mary's Hospital Medical School, London.
Gastroenterology	17 & 18.	Dr M. B. Pepys, Royal Postgraduate Medical School, Hammersmith Hospital, London.
Rheumatology	19 & 20.	Dr G. R. V. Hughes, Royal Postgraduate Medical School, Hammersmith Hospital, London.
Transplantation	21.	Mr J. E. Castro, Royal Postgraduate Medical School, Hammersmith Hospital, London.
Immunology of Cancer	22.	Mr J. E. Castro, Royal Postgraduate Medical School, Hammersmith Hospital, London.
Immunosuppression	23.	Dr J. G. McVie, University of Glasgow, Glasgow.
Myasthenia Gravis	24.	Dr W. J. Irvine, Royal Infirmary, Edinburgh.

The registration fee up to January 31, 1979 is £65 (excluding accommodation and meals) and £80 thereafter. Accommodation will be available at the University Halls of Residence, but if alternative accommodation is required it should be booked direct Regstration form, further particulars and enquiries should be addressed to:

Dr W. J. Irvine, Endocrine Unit and Immunology Laboratories, Royal Infirmary, Edinburgh EH3 9YW (UK).

8575(D)

London School of Hygiene and Tropical Medicine and The Royal Postgraduate Medical School (University of London)

MSc in Medical Microbiology

Advanced academic and practical training in bacteriology, virology, immunology, mycology, microbial genetics and medical parasitology as applied to clinical and preventive medicine is provided through an intercollegiate course of study extending over two years.

The first year will be spent at the two Schools of the University followed by a second year at a recognised laboratory. Candidates with satisfactory evidence of prior supervised practical training may be exempted from the second year of study.

The course is open to medical, dental and veterinary practitioners, graduates in science or pharmacy and other suitably qualified students.

Exemption from the Primary Examination for the MRC Path may be granted to successful candidates. The course is accepted as recognised training for College examinations.

A limited number of Medical Research Council awards may be available to suitably qualified British subjects resident in the United Kingdom.

Closing date for applications: 1 March, 1979.

Further particulars from the Registrar, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT.

8513(D)

ASSISTANTSHIPS

ENVIRONMENTAL SCIENCE/CHEMISTRY

Teaching, Research Assistantships for graduate study in M.Sc. and Ph.D. program. Specialisations include aquatic and atmospheric chemistry. Write to Dr Raymond Lutz, Dept. of Chemistry, Portland State University, P.O. Box 751, Portland, OR 97207 USA. Equal Opportunity Employer.

8580(P)

GRANTS

UNIVERSITY OF LONDON GRANTS FOR RESEARCH

Applications are invited from members of the University and teachers in Schools of the University for grants from the Central Research Fund to assist specific projects of research and for the provision of special materials and apparatus. Grants are not made for normal maintenance. Applications must next be received not later than December 15, 1978. Forms of application and further particulars may be obtained from the Secretary to the Central Research Fund, University of London, Senate House, London WC1E 7HU.

8536(H)

UNIVERSITY OF BRISTOL

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All five courses qualify for a quot of S.R.C. advanced course studen ships. Further details of the individu courses may be obtained from th Head of the Department concerne University of Bristol, BS8 1TH. Enquiries are also invited for details o opportunities for research leading t the degree of Ph.D. 8568(D)

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DIAGNOSTIC METHODS IN CLINICAL VIROLOGY
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The Course will be run by Dr A. Smith and J. Bertrand, and wi consist of lectures, demonstrations an practical exercises involving Ce Culture, Preparation of Clinical Spec mens, Complement Fixation, Haemag glutination, Immunofluorescence Radial Haemolysis, Virus Neutralisa tion, Electron Microscopy, Radioin munoassay and Enzyme Immunoassa

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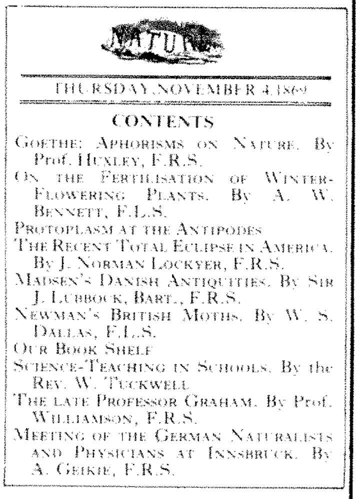

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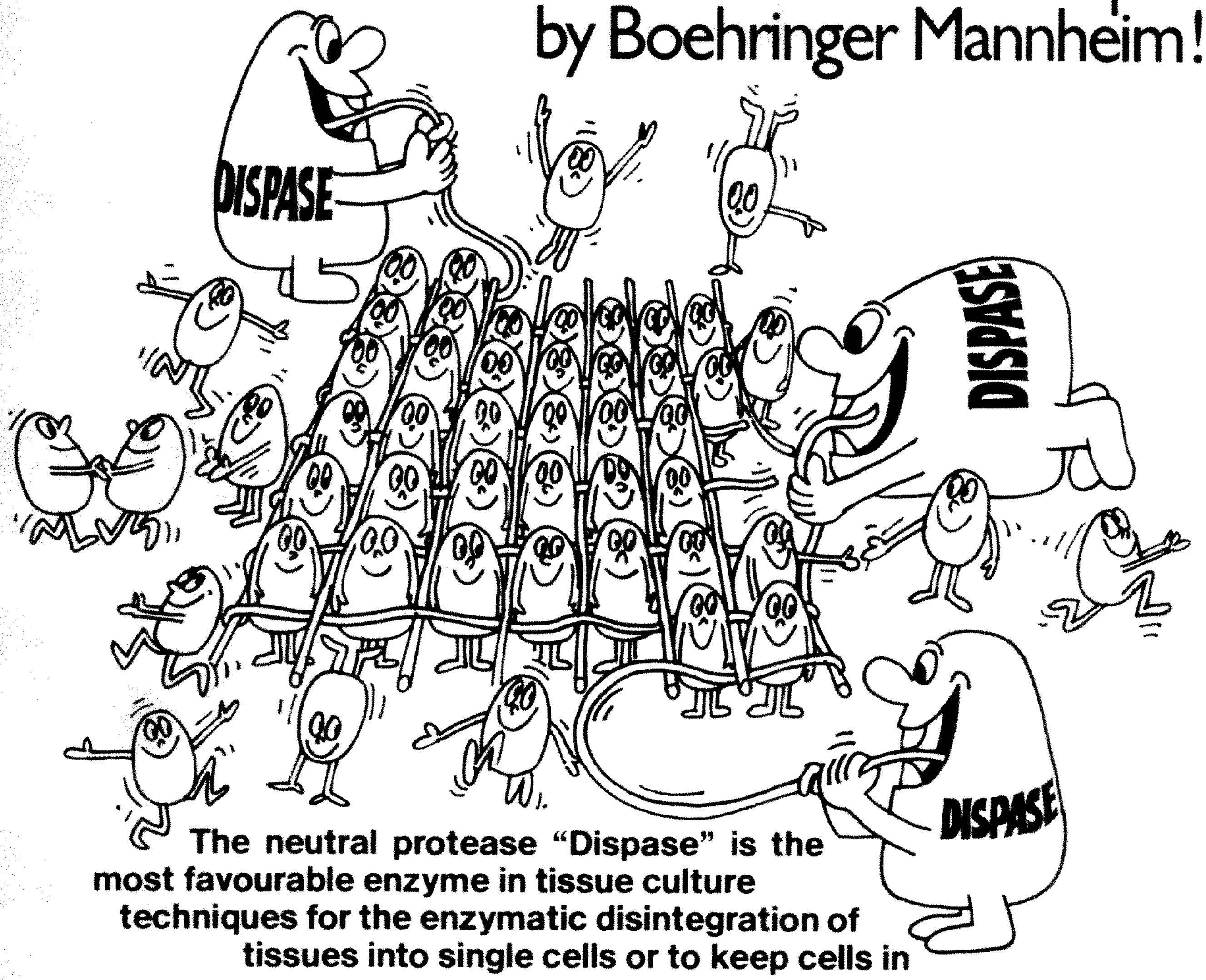

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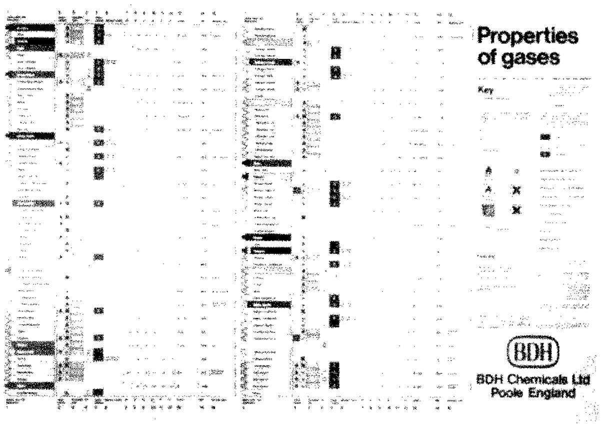

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(i) only one reference number need be used if the reference is to several papers by identical authors.

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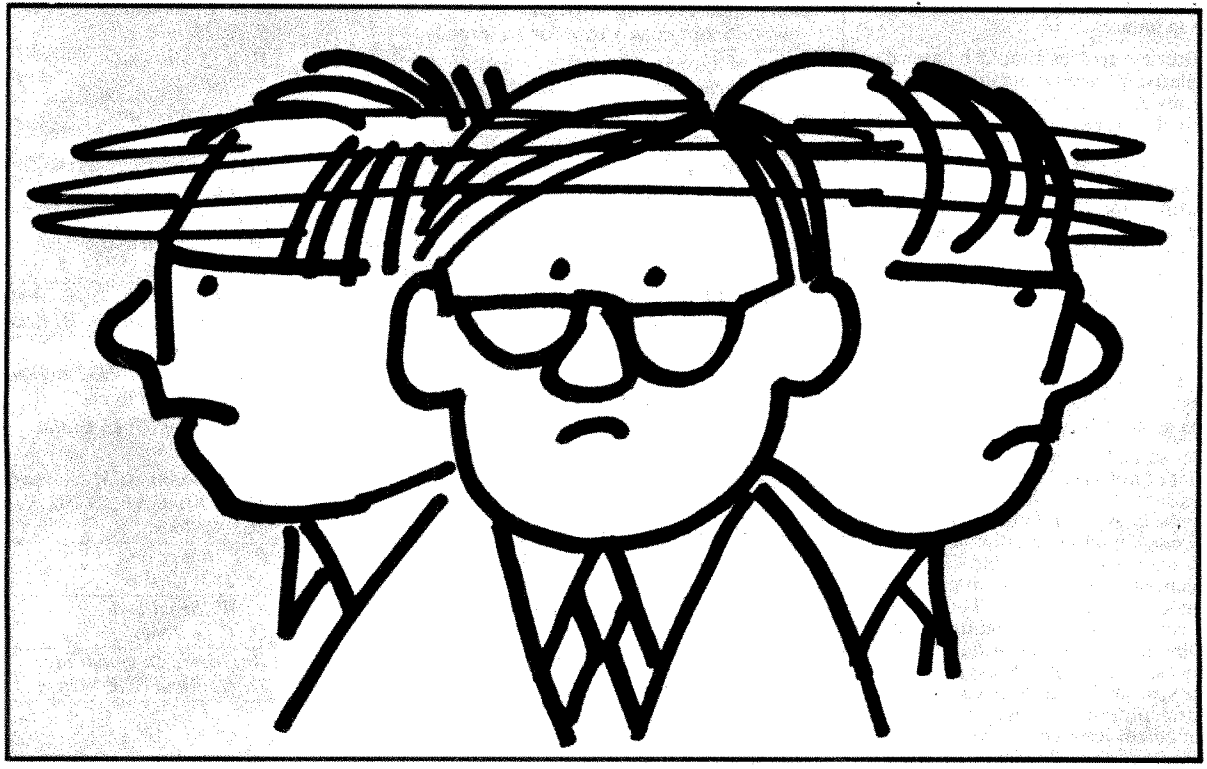

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Rothschild's numerate arrogance

Lord Rothschild, speaking on British television last week, argued that we should develop a table of risks so we could compare, say, the risk of our dying in an automobile accident with the risk of Baader-Meinhoff guerillas taking over the nuclear reactor next door. Then we would know how seriously to take our risks, be they nuclear power, damage to the environment or whatever. The 'econuts' (Lord Rothschild's usage) would soon be put in their place if we put numbers to the risks they envisage. What could be more reasonable than that? Has Rothschild lightened the darkness of the risk business with the light of science and number?

No. It is fine for Rothschild to demonstrate his agility with arithmetic, converting probabilities from one form to another (and implying that the viewers could not do it) but this is only the kindergarten of risk. He gave only the briefest of hints that there might be more to it when he called for errors to be quoted with risk estimates and when he remarked—in a brief aside—that occasionally one had to rely on expert assessment of risks, as in the case of nuclear reactors. But he threw these cautions away when he quoted not a single error on the risks he enumerated, nor developed the problem of the partisan 'expert'.

More than this, Rothschild confused two fundamental distinct kinds of risk in his table: known risks—such as car accidents—where the risk is simply calculated from past events; and unknown risks—such as the terrorists taking over a fast breeder—which are matters of estimating the future. The latter risks inevitably depend on theory. Whether the theory is a social theory of terrorism or a risk-tree analysis of fast breeder failure, it will be open to conjecture. And it ought to be remembered that the history of engineering is largely a history of unforeseen accidents. Risk estimates can be proved only by events. Thus it is easy for groups, consciously or unconsciously, to bend their calculations to suit their own objectives or prejudices. With unknown risks it is as important to take these into account as to come up with a number. Hence the danger of relying on 'experts' who are committed to a particular future. What is needed more than an expert table of risk values is a democracy of risk assessment, where the basic information on which conjectural risks are calculated is made available to as many groups as possible.

Moreover some future risks are largely incalculable because we have no theory. What is the risk, for example, of a fast breeder economy leading to an erosion of civil liberties? This is a matter of legitimate concern, but how can Lord Rothschild's table deal with it? It is a conceit among some scientists that scientists alone are rational, and Lord Rothschild projected this conceit particularly clearly.

Peter Chapman, Director of the Open University Energy Group, caricatured this view when he spoke to the Parliamentary Liaison Group for Alternative Energy Strategies last week: "'rational' things, facts, are concerned with the reproducible aspects of the world. In the energy business this means the efficiency of machines, the heat loss from houses, the number of houses and so on. If we agree on the meaning of these terms we can agree how to measure them and hence have a basis for agreeing what to do. Anything that cannot be measured (and this included all my emotional reactions to things, people, and relationships) has to be left out. It is dubbed 'irrational' and excluded."

Science and numeracy are undoubtedly important parts of rationality, but they are by no means the whole of it; and scientists do a disservice to both by pretending that they are. In part they fail to distinguish the process of science from the product. Science in the making—like a projected risk—is always conjectural and hypothetical; it is confirmed by repeatability (part of the philosophy of a democracy of risk) and acceptance within the scientific community (a social event). It is never a matter of 'experts'; that is mere scholasticism. Future risks must be science in process for there can—by definition—be no experiment.

It is objective and rational to take account of imponderable factors. It is subjective, irrational, and dangerous not to take account of them. As that champion of rationality, the philosopher Bertrand Russell, would have argued, rationality involves the whole and balanced use of human faculty, not a rejection of that fraction of it that cannot be made numerical. By all means let us have numbers, where they can be agreed upon; but let us not be mesmerised by them. □

US to increase public participation in regulation of DNA research

SIGNIFICANT changes in procedures for regulating research involving recombinant DNA techniques are soon to be announced by the US National Institutes of Health (NIH). In particular, there will be greater public participation in both local biosafety committees and the NIH's Recombinant DNA Advisory Committee; and tighter provisions determining responsibility for ensuring that regulatory guidelines are observed.

Other changes expected to be announced are expansion of the scope of the present guidelines to cover recombinant DNA research carried out in institutions receiving support for such research from any federal agency (not just NIH); stricter rules about the public notice required to alter the guidelines; and increased provision for taking "risk assessment" considerations into account when deciding whether certain types of experiment should be exempt from the guidelines.

A revised version of the guidelines, which were first introduced by NIH in July 1976, were delivered to Secretary for Health, Mr Joseph Califano, at the end of last week. If he approves the changes, as is expected, then the new guidelines will be published in the *Federal Register* within a few weeks.

Proposed revisions to the guidelines were issued by Dr Donald Fredrickson, director of NIH, earlier this year. In particular, Dr Fredrickson has recommended lowering the levels of containment required for certain types of experiments (and to exempt other experiments from the guidelines completely) in the light of scientific evidence that has accumulated since concern over the possible social impact of recombinant DNA research was first raised five years ago.

Most of the suggested revisions to the scientific aspects of the guidelines are expected to be retained in the final version, apart from a few deletions from the list of exemptions. The major changes will occur in Part IV of the guidelines, that which covers "roles and responsibilities".

Much of the impetus for this change has come directly from Mr Califano, responding on the one hand to pressures from Congress—which is still threatening to introduce legislation to make up for any deficiencies it perceives in the guidelines—and on the other to public interest and environmental groups, which have been consistently critical of what they see as attempts to retain decision-making within the scientific community.

When NIH's proposed revisions were published, Mr Califano added an introduction specifically requesting comments on the proposed mechanisms for administering and revising the guidelines. Many such comments were aired at a public meeting held in Washington in September before a departmental review committee established by Mr Califano under the chairmanship of Mr Peter Libassi, chief counsel of the Department of Health, Education and Welfare.

According to sources in Washington, the major impact of the review committee's intervention and its study of the various comments and criticisms received, will involve aspects of public participation in the regulatory process, as well as channels of responsibility and accountability.

In his proposed revisions to the guidelines, for example, Dr Fredrickson had suggested that considerable authority for the conduct of recombinant DNA experiments be devolved from NIH to local biosafety committees; and that, in addition to including those with expertise in the relevant fields of science and public policy, at least one member of such a committee "shall not be affiliated to the institution."

Critics, such as Friends of the Earth and the Environmental Defense Fund, argued that this did not go far enough in involving the public in the regulatory process, in particular since the wording still allowed the external appointment to be made by the institution. The final version is expected to say that at least two members—or 20% of the total—should be appointed from outside, for example from environmental groups or local or state safety bodies.

Furthermore, membership of the Recombinant DNA Advisory Committee, another target of criticism, is to be increased from 14 to 20; the number of "public interest" representatives, is expected to be increased from two to six.

Additional changes to NIH's suggestions in the final version of the guidelines include clearly stated procedures for the director of NIH to adopt if it is proposed to make changes in the guidelines, specifying which changes need to be offered for public comment, which require advance notice, and which can merely be announced. Provision is also expected to be included for NIH to be able to remove the authority delegated to a local biosafety committee if it considers the committee's performance to be unsatisfactory.

One target of criticism in the suggested revisions was the proposal that, although certain types of experiments would continue to be prohibited, case-by-case exceptions would be allowed for experiments for which there were "compelling social or scientific reasons", provided that weight was given to "scientific and social benefits and to potential risks".

This language is now to be tightened up, so that the general standard for all such decisions will be an assessment that the experiment in question poses "no serious risk".

Two final points relate to the commercial aspects of recombinant DNA research. Firstly, the advisory committee is to be asked to consider what ground rules should apply to experiments involving more than 10 litres of culture containing altered genes.

All such experiments—not merely those involving "harmful products," as at present—would be prohibited, but still subject to waiver by the director of NIH; in commenting on this ban, the pharmaceutical company Eli Lilley told Dr Fredrickson that the necessity for such experiments was "imminent".

Secondly, reflecting a concern that a "voluntary" approach to regulating experiments in private industry suggested by NIH is likely to prove inadequate, the Food and Drug Administration is to consider whether similar guidelines should be applied directly to the pharmaceutical industry.

Many of the expected changes are likely to be welcomed by public interest and environmental groups, since they go some way towards meeting the criticisms of the guidelines on which such groups have been concentrating in recent months. "Any movement towards more responsible mechanisms for operating the guidelines is a step in the right direction," Dr Jonathan King, professor of biology at Massachusetts Institutes of Technology said last week.

Officials at NIH are said to be less happy, fearing that they are being pushed into a more regulatory role than many would wish, and are losing some of their flexibility to manoeuvre. Publication of the revised guidelines is thought to have been delayed by negotiations between NIH and DHEW.

Many of the changes answer criticisms given by Senator Adlai Stevenson, in a letter to Mr Califano last month, as reasons why he considered legislation still necessary. Mr Stevenson is said to be happier with the final version and the prospect of legislation may be receding.

David Dickson

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US and Japan to cooperate in fusion and high energy physics

A BROAD programme for collaborative research in a number of areas of energy science has been agreed in principle by the United States and Japan. The programme, which will include fusion research and high energy physics, is expected to involve expenditure of about $1,000 million by the two countries over the next ten years.

The main areas of collaboration were agreed during a visit to Japan in September by Dr James Schlesinger, Energy Secretary, and Dr John Deutch, head of the Department of Energy's office of energy research. The details were worked out in a series of meetings held in Washington last week; and it is hoped that an agreement will be signed within four months.

The two main items in the programme are research into magnetic fusion, and into liquefaction of coal. In addition, the two sides have agreed to cooperate in solar photovoltaics, geothermal energy, and high energy physics.

As far as fusion is concerned, the main focus of Japanese interest will be the General Atomic Company's large Doublet III programme in San Diego, to which the Japanese are expected to contribute about $50 million.

In addition, the two sides have agreed to set up a joint research institute for fusion research. The Japanese end of the institute will probably be in Nagoya; no decision has been reached on a US site, with Massachusetts Institute of Technology and the University of California at Los Angeles being possible contenders.

In return for being allowed to participate in the fusion programme, the Japanese have agreed to contribute 25% of the costs of a $700 million coal conversion demonstration plant planned for construction by Gulf Oil in West Virginia. The German government has already agreed to contribute a further 25%.

As for high energy physics, the two sides have in principle agreed on joint research and development into accelerator and particle detection technology and the joint construction and use of new facilities. It has been suggested for example that the Japanese may contribute to enhancing the 400 × 400 GeV intersecting storage accelerator (ISABELLE) at the Brookhaven Laboratory on Long Island. In the longer term, many US physicists are hoping that it may be possible to obtain joint funding for the next generation of particle accelerators. **David Dickson**

Home insulation may increase radiation hazard

SCIENTISTS at the University of California's Lawrence Berkeley Laboratories have suggested that the reduced ventilation in private houses resulting from conservation measures may pose a potential health hazard, by increasing exposure to low levels of the radioactive gas radon.

Radon-222 is produced as part of the decay chain of uranium-238. Both the gas and its short-lived decay "daughters"—in particular the alpha-emitters polonium 218 and 214—are present in the natural environment and are a recognised source of background radiation. Furthermore, radon's precursor, radium-226, is a trace element contained in most rocks and soil; and indoor radon sources therefore include many building materials.

The health hazard is caused principally by radon's daughters, which can attach themselves to airborne particles and if inhaled, can lodge in the lung and emit short-range alpha radiation. In recent years, the hazardous aspects of high-level radon exposure have been dramaticaly highlighted by the high incidence of lung cancer among uranium miners. And only recently the federal government has been taking steps to minimise public exposure to the radon emitted by uranium mill tailings.

Six scientists at the Lawrence Berkeley Laboratory's energy and environment division have now suggested that, if medical data from the uranium miners is extrapolated backwards—and assuming a no-threshold, linear dose-response relationship at low levels of exposure—the decreased ventilation in private homes achieved in the interests of energy conservation could theoretically lead to an extra 20 to 200 annual deaths per million population from lung cancer.

The scientists point out that despite recent interest in the effects of radon exposure in both Sweden and the UK, there is still little detailed knowledge about the effects of low level exposures over long periods of time, and that any conclusions are highly speculative. "We are not issuing a warning, but merely calling attention to a possibility that should be watched and studied", says Dr Craig Hollowell, one of the scientists concerned.

Despite this, a report produced by the scientists states that: "It is likely that some increased lung cancer risk would result from increased radon exposures; hence it is desirable not to allow radon concentrations to rise significantly".

In the long term, the scientists suggest that it may be necessary to include radon exposure levels in building standards. In the short term, they suggest a number of measures—such as sealing walls and floors, or coupling mechanical ventilation with heat exchangers—to reduce radon exposure.

The scientists have applied to the Department of Energy for further funds to carry out a more detailed investigation of the potential hazards—a request which has fallen between the department's environmental health and conservation responsibilities.

Some money has already been granted to study the radon emission of building materials. A complementary proposal for studying the health effects is being considered by the department's office of energy research. No one is yet prepared to admit that increased radon exposure does pose a health hazard, however small; but with growing concern about the long-term effects of low level radiation, no one is yet prepared to dismiss the hypothesis as unfounded. **David Dickson**

New Spanish Constitution leaves science policy open

THE Spanish Parliament has just voted by a large majority (more than 90%) for the second democratic Constitution of this century. On 6 December the Spanish people are called to vote the text in a referendum. The main political forces, from extreme left to neofrancoists, will campaign for its approval.

Science is mentioned twice in the first part of the new Constitution. Article 20 recognises "the right to literary, artistic, scientific and technical production and creation" and article 44 says that "the public powers will promote science and technical and scientific research for the benefit of general interest".

The mention of research in the articles dealing with the organisation of the State may be of greatest significance for scientists. One of the central constitutional problems has been the formation of autonomous governments in different parts of Spain. Article 148 lists the matters transferable to autonomous communities and it includes "the promotion of culture and research". However "the promotion and general coordination of technical and scientific research" can be found in the list of matters reserved

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for central government. Nevertheless anything is possible because article 150 says that the central government may transfer or delegate to autonomous communities matters attributed to it.

The reason why the organisation of research is mentioned in the Constitution comes from the present unequal distribution of research centres in Spain. The question is left open because some people think that if control over research is given to autonomous governments research would be more balanced and related to the needs of different areas; others fear that it could be more difficult for a smaller community to build an appropriate system of financing research and that coordination may not be easy.

Parliament will have to make a decision on these issues within the next few months. From a practical point of view it is very important that decisions are made quickly as the present provisional situation in science makes work very difficult. For instance one of the main sources of research funds (FNDICT) may disappear next year and laboratories have not yet received the credits corresponding to applications made in 1977.

What remains to be seen is if political decisions will be taken soon enough to prevent the closing of certain laboratories and if the problems are treated with sufficient imagination to achieve an adequate structure for research.

Pedro Puigdoménech

Sweden's new government wrestles with nuclear power

NUCLEAR power has finally proved to be the undoing of Sweden's first non-socialist coalition government in 44 years. Former Prime Minister Torbjörn Fälldin handed his resignation to the Speaker on 5 October.

The government fell because some members of Fälldin's Centre Party opposed the coalition's decision on the loading of two reactors: Ringhals 3 and Forsmark 1. Under the Stipulation Law, the reactor owners could be given permission to load only after they had secured an acceptable reprocessing contract. They also had to show how and where the highly-radioactive waste resulting from reprocessing could be finally stored. Judging the application by these criteria, the government found "that the preconditions for consent are deficient in one respect. The application cannot therefore be approved".

The government went on to say that in order for the application to meet the conditions laid down for secure final storage, there should be test drilling to "show the existence of a sufficiently large rock formation of appropriate depth" with the qualities already prescribed by the nuclear industry's Nuclear Fuel Safety (KBS) project*. If the applicants found rock they considered suitable and applied again, the government would ask the Nuclear Power Inspectorate to judge the application. If the Inspectorate approved it, the government would give permission for the reactors to be loaded.

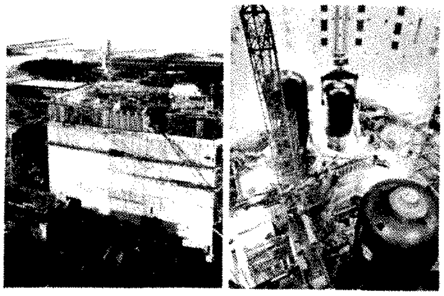
This agreement was immediately interpreted by the Press as a 'soft yes' to the loading, as it was assumed that the test drilling was a mere formality which would quickly produce the required rock. The Centre Party was criticised for yet another capitulation: although anti-nuclear, it had made several compromises on the issue during the government's term. How much influence these attacks had is uncertain, but, at the insistence of former Energy Minister Olof Johansson and other members of the Centre Party, Fälldin demanded that his coalition partners should agree to a referendum on the issue. They refused, and the government fell.

After much speculation, a minority Liberal party government was sworn in on 18 October. The new Energy Minister, Carl Tham, favours nuclear energy, but his statements since becoming Minister have been cautious.

Tham has said that the investments already made in nuclear reactors should not be wasted, and that those already in operation or being constructed should be used if security demands can be met. This would mean drawing the nuclear line at eleven reactors. He also wants to tighten up security on all energy forms. The new government will, he says, follow the old one's agreement on the Ringhals 3 and Forsmark 1 reactors.

Since the government's fall, debate has raged over the extra drilling requirements. Will they prove to be a simple formality, or was this a subtle way of delaying the two reactors perhaps for years? Engineer Lars Bertil Nilsson, chief of the KBS project, is confident that suitable rock will be found quickly. "There are certainly many places in Sweden where the rock is suitable", he says. "According to the government's statement, we simply have to find one of them. We don't need to choose any particular place now for final storage: that's a long-term business and will need to take a lot of other things into account besides the rock itself: buildings, transport, public opinion, etc. All we have to do now is to show that suitable rock exists. It is our judgement that we need only drill four new holes at Finnsjön, near Forsmark, and three new ones at Karlshamn. The drilling has just begun and will be finished by the end of the year. Then we'll put our report together and make a new application to the government in 1979."

His optimism is not shared by various researchers and some of the geologists actually doing the drilling. They have claimed that reliable results cannot be expected before two to ten years.



Forsmark 1 and Ringhals 3 under construction.

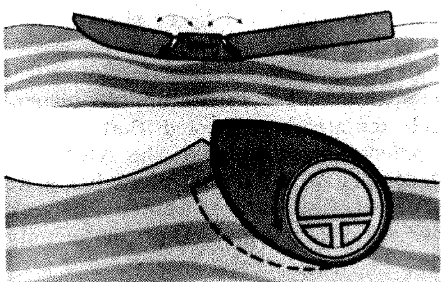
Meanwhile, the Nuclear Power Inspectorate is preparing itself for the new application. According to Dr Thomas Johansson, Vice-Chairman of the Inspectorate's Board, an expert panel of geologists is being set up to review the application. Before it is presented, however, the board is asking the panel to discuss guidelines for its evaluation. The panel should list the qualities that the nuclear power industry has said suitable rock should have; discuss possible measuring techniques that could be used to claim that the rock actually has the required characteristics; and decide what constitutes "showing" that the rock is suitable. "None of the geologists on the panel is involved in the drilling", says Dr Johansson. "The intention is to have a completely independent review."

What, then, of the future? "I am rather pessimistic", says anti-nuclear activist Björn Gillberg. "The Centre Party committed suicide when it passed the Stipulation Law. Whether or not we want nuclear energy should be decided on very broad grounds: social, political, psychological, environmental, international. To reduce it to the technical handling of waste, as the Law does, simply turned out to be a trap. The Centre Party didn't realise it had set a trap for itself until it was too late. They're nice guys, but they're naive. I can see the [pro-nuclear] Social Democrats winning next year's elections."

Wendy Barnaby

**The first KBS report, against which the Ringhals 3 application was judged, is called "Handling of Spent Nuclear Fuel and Final Storage of Vitrified High Level Reprocessing Waste". It is available from KBS, Brahegatan, S–102 40 Stockholm.*

Enthusiasm for UK wave power survives high costs



The Cockerell raft (top) and Salter duck

WAVE energy is not the white knight rescuing the fair society. That seems certain. Nevertheless the participants at a conference on the UK wave energy programme in London last week were optimistic about their ability to solve eventually the formidable problems of harnessing the power of the waves through steady work. And they were equally optimistic about the value of doing so.

The bad news about wave energy is that estimated costs per kW installed for the UK are £5,000-£9,000 compared to £500-£1,000 for a fossil or nuclear station. Current devices are a factor of 20 too costly for the value of the energy they can produce, concludes a report prepared by the consulting firm of Rendell, Palmer and Tritton in conjunction with Kennedy and Donkin. Referred to as a 'hatchet job' by observers outside the wave energy programme, the RPT report, commissioned by the Wave Energy Steering Committee, does not appear to have lowered morale. As Dr Stephen Salter of Edinburgh University, designer of one of the principal devices, the Salter duck put it: "Sir Hermann Bondi (Chief Scientist in the Department of Energy) has given us the best wings money can buy. RPT has told us how hard the ground is and the rest is up to us." Clive Crove Palmer, Programme Director of Harwell's Wave Energy Group told the conference: "This group thrives on problems and challenge. They won't just lick them, they'll eat them."

The high cost of the early designs arises from the diffuse character of the waves themselves. To capture the energy, large areas of device have to face the on-coming waves. With either concrete or steel the designs have been expensive. "But remember", says Grove-Palmer, "the Wright brothers' first estimate of the cost of air travel was $30,000 per mile and they went right ahead".

The UK is favoured with some of the best off-shore sites in the world for the exploitation of wave power. Energy in waves is governed by wind strength and length of water over which the wind blows. Prevailing Westerly winds blowing across as much as 1,000 miles of the North Atalantic create waves of average power of 50 kW per metre of wave front.

Since the UK wave power programme is only two years old and since the conference participants have an obvious enthusiasm for what has been accomplished with the first generation devices, Grove-Palmer's optimism may be justified. What, then, has been achieved in two years?

Two of the main devices are the Cockerell raft and the Salter duck. The Cockerell raft uses the relative angular motion of two segments of raft and the Salter duck uses a similar action of oscillating pods about a common spine. The precise mode of conversion is a subject of study. The rocking action can be used to drive a pump which can pressurise a fluid which can drive a turbine. But other proposals include on site conversion to hydrogen, on site desalination or direct use of the mechanical power to make heat.

Other devices under active consideration are the oscillating water column (OWC) and the Lancaster University flexible bag. The OWC is a box with a hole in the top. The waves make the water level oscillate and air is forced through the hole to drive a turbine. The flexible bag is potentially the cheapest design. Air filled bags are attached to a submerged hull. The wave crests collapse the bags and the displaced air drives a turbine. The bags are filled during wave troughs from a low pressure duct.

Grove-Palmer detailed six areas of future work in his closing summary.

- The need for better wave data. The irregularity of the waves in height and direction needs study. According to M. J. Platts of Wavepower Ltd, the builders of the Cockerell raft, one difficulty is that the oil industry has kept its accumulated wave data from the North Sea platforms 'inaccessible'. Platts has been unable even to arrange a visit to one of the platforms.
- Detailed critical engineering studies of how best to extract the power.
- Spine articulation of Salter's ducks. The bending moments are severe.
- Tests of the oscillating water column in real sea conditions.
- Further tests of the flexible bag because of its low cost.
- Mooring. Existing mooring equipment has been designed for ships and is unsuitable. Equipment is needed to withstand powerful mooring forces in high seas, corrosion and fouling by marine organisms over a period of 25 years without inspection. (Ships get drydocked every three or four years).
- Size and survival in high seas.

Aside from the technical problems are the problems of actually producing a large scale operational system. M. J. Platts estimates that all the design problems need to be solved and a fully operational production system needs to be built before 1990 if there is to be significant (20%) wave power production by 2020. After 25 years, production must be entirely given over to replacement so building up sufficient capacity before that time is a problem. Platts also points out that on this time scale one can expect significant social and economic changes which are impossible to predict.

A novel feature of the wave energy programme is the attention now being paid to environmental and social aspects. Preliminary estimates indicate that the effect of wave energy devices 10 km off shore will be to increase the size of beaches and the economic well being of the local population. But the lobster, herring and the £20,000,000 salmon industry are concerned that installation of the devices could interfere with the normal migration and mating behaviour of the fish. A third nuisance could be the possibility of seals finding the devices habitable with unknown consequences.

Problems of interfering with shipping are more serious. There are problems of collisions, the generation of rougher seas by wave reflections from the devices, and the need for reliable warning lights and radar reflectors to mark gaps up to 2 km wide to a standard much higher than ever attempted in the open sea.

To many wave energy is an attractive alternative energy possibility. "There is still a major amount of work ahead before conclusions can be reached about the future of the technology" said Dr F. J. P. Clarke, Chairman of the Wave Energy Steering Committee. But realism must tell us that any talk of a full scale or near full scale wave energy station out at sea by the early 1980s is premature. We still have to evolve concepts and engineering designs that would justify the major expenditure involved in such a step." Clarke tempers his judgement with a more positive note. "The resultant [wave energy] team . . . comprises a substantial national asset, and given continuous government support it is determined to find solutions. The natural resource is there and if anyone can learn how to capture it this team will."

Joe Schwartz

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news in brief

Pochin calls for improvements in safety at Aldermaston: Substantial improvements in safety measures are urgently needed at the Atomic Weapons Research Establishment, Aldermaston, according to Sir Edward Pochin in his recent report on safety at AWRE. Workers there should be better protected from inhaling radioactive dust, the report says.

Pochin's inquiry, leading to the recent report, was set up following events last August when three women from the special laundry at AWRE were found to have plutonium in their lungs in excess of the level recommended by the International Commission on Radiological Protection.

In the report, Sir Edward says that the general level of health protection in the plutonium processing buildings is "of borderline adequacy", even though the industrial safety record is excellent.

Cutbacks in the number of safety staff and inadequate training had caused a serious decline in monitoring of radioactivity, he says, and, consequently, workers were being exposed to potentially dangerous levels of radioactivity. Monitoring standards had fallen behind those in other establishments in the UK and the US, although an increase in the number of personal air samplers, and a body counter to estimate plutonium in the lung, now being built, would improve matters.

Sir Edward recommends that in the long term major improvements should be made to buildings where solid and liquid waste are reprocessed; the containment of alpha-emitting radionuclides is very difficult with the present safety measures, he says.

AWRE and the National Radiological Protection Board have set up a joint committee to review the radiation exposures of individual workers, and inspectors from the Health and Safety Executive are to visit the establishment.

Supreme court rejects Podrabinek's appeal: Aleksandr Podrabinek (pictured right), the Moscow paramedic sentenced last August to five years internal exile last week had his appeal rejected by the Supreme Court of the Russian SFSR. The court stated that the charges of anti-Soviet slander had been upheld.

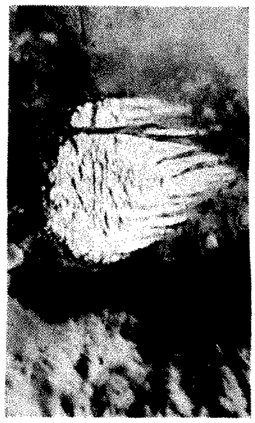
Mr Podrabinek, an active member of the illicit "Working Commission for the Investigation of the Use of Psychiatry for Political Purposes" is the author of a 265-page *samizdat* dossier on Soviet abuses of psychiatry to crush dissent.

Mr Podrabinek was not permitted to attend the appeal hearing. Nor was his chosen defence counsel, the London lawyer Louis Blom-Cooper. According to Mr Blom-Cooper Podrabinek's original trial involved the violation of no less than 78 provisions of Soviet legal procedure. Dissident sources in Moscow suggest the same pattern was followed at the appeal hearing—no defence witnesses were called nor was Podrabinek's own statement read to the presiding judges. *(Photo courtesy of Keston College.)*

Patent rights for university researchers: In an attempt to clear up the issue of whether patent rights for inventions made by researchers in British universities should be held by the researcher or the university, the Association of University Teachers (AUT) has sought the opinion of a leading firm of solicitors, which has said that the invention belongs to the researcher provided that he or she was not employed specifically to invent.

After the 1977 Patents Act, the Committee of Vice-Chancellors and Principals issued a report on the ownership of patents which tentatively suggested that the individual should hold patent rights. However, the AUT says that in the past most compensation for inventions has gone to the universities. The AUT, therefore, wanted "to seek an independent view", said Mr John Akker, its deputy general secretary.

The AUT hopes soon to begin talks with the Committee of Vice-Chancellors and Principals to lay down guidelines on the ownership of patent rights which could be applied to all British universities.



NASA is to investigate Martian "White Rock"; This strange, white, horseshoe crab-shaped feature found inside a Martian crater (see photo) is to be studied by scientists working with the NASA Viking mission. The feature, nicknamed 'white rock', is the only one of its kind on Mars. Its composition is unknown, but it is unlikely to be either ice or snow because it lies near the equator.

The 'white rock' is about 18 km long and 14 km wide, and is at the bottom of a crater located at 8° South, 335° West.

Women scientists still lag in US: Despite significant increases in the proportion of women science graduates emerging from US colleges and universities, their salaries are still lower than those of men with comparable training and experience at every age, every degree level, in every field and with every type of employer (except for chemistry BAs employed by industry), according to figures released last week by the US Scientific Manpower Commission.

The commission says that detailed statistical analysis of the participation of women in the professional labour force indicate that women have roughly doubled their proportion of earned degrees in the sciences since 1970, and quadrupled their share of engineering bachelor's degrees. However, unemployment rates for professionally trained women continue to be two to five times higher than for men in the same field with the same level of training, and the gap increases at higher levels.

In the federal government, women still lag well behind their male counterparts in grade level (and hence salary). Thus among the federal government's 1,700 microbiologists, the average salary is $23,260 for men and $18,550 for women.

New parliamentary group for alternative energy: The UK Parliamentary Liaison Group for Alternative Energy Strategies called its first public meeting last week at the House of Commons. Chaired by Frank Hooley, MP, and supported by many environmental groups, the Group called Sam Berman, Director of the Lawrence Berkeley Laboratories, Peter Chapman, Director of the Open University Energy Group, and Gerald Leach, of the International Institute for Environment and Development, to speak. The next meeting is on 29 January with Tony Benn, Secretary of State for Energy.

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Health and safety research gets underway

The UK Health and Safety Executive (HSE) last week published its research report for 1977—the most comprehensive yet to be published on the executive's research activities. **Alastair Hay** here looks at the research labs run by the HSE.

FEW research institutes would complain about a yearly budget increase of more than 40%; fewer still would be able to approach this level. But last year the HSE spent £8.5m on 'research' testing, and scientific support activities", compared to £6m in the previous year. There is, however, good reason for the executive's windfall, as the 1977 research report makes clear. The HSE is a relatively new institution, brought into being by the 1974 Health and Safety at Work Act, and so is still growing.

The Act established a Health and Safety Commission (HSC), a body with the task of determining policy for health and safety in Britain. A tripartite organisation consisting of trade union, employer and local government nominees, the commission has a role which can be compared with that of a company board of directors, with the basic difference that of all its members, only the chairman is a full-time employee. The HSE was formed to implement the commission's decisions, and is responsible for the day-to-day policing the HSW Act.

All people at work—with the exception of domestic workers in private employment—are covered by the Act. The HSE required facilities from several of the more established ministries in order to carry out its obligations. The Alkali and Clean Air Inspectorate was transferred from the Department of the Environment, a move vigorously opposed by the DoE. Other transfers, from the Departments of Energy, Employment, and, Prices and Consumer Protection and the Ministry of Agriculture, Fisheries, and Food, were achieved more easily.

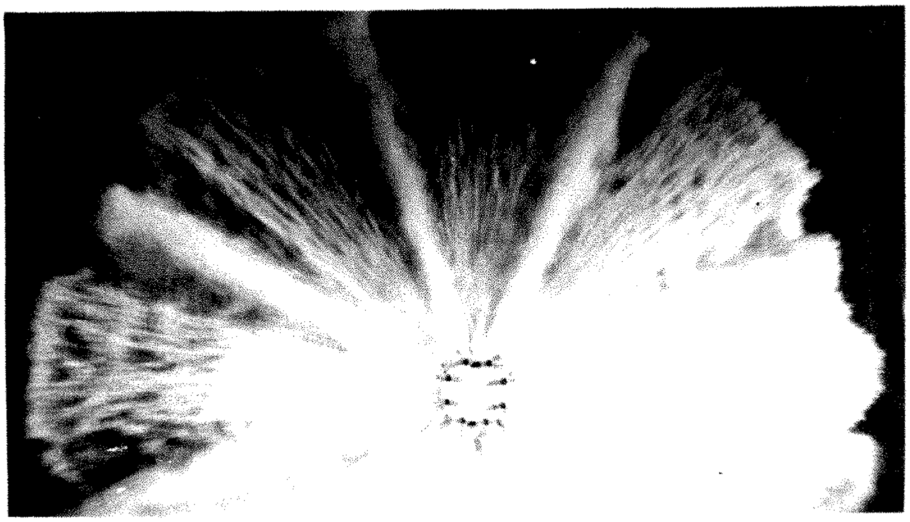
It is hardly surprising, therefore, that the HSE was much involved with its own organisation during 1975 and 1976. The report indicates that 1977 was the first year that the HSE could concentrate its efforts on new areas of research, a situation reflected in its budget. It is also pointed out that some 28% of the £8.5m was allocated to extramural work, with the aim of providing the executive with additional expertise and facilities not immediately available to it.

These are the Health and Safety Laboratories (HSL) run by the HSE. The Explosion and Flame Laboratory (EFL) is based at Buxton; the Occupational Medicine and Hygiene Laboratory at Cricklewood; and the Safety Engineering Laboratory in Sheffield.

Explosions and flames

Dr Herbert Eisner is the Explosion and Flame Laboratory's director. Outlining the work carried out at Buxton, he indicated that much of the research is concerned with the development of test methods, pointing out that "If a hazard is present, you need to discover the mechanism which caused it".

Of particular concern to EFL are hazards encountered in gases. The reason behind the EFL's interest in gas is obvious from its history. It started life over sixty years ago as the Safety in Mines Research Establishment and in an even more remote location, in Cumberland. From its inception, the laboratory has been concerned with the prevention of explosions in coal mines and the development of safe electrical equipment for use in explosive atmospheres. Research of this nature has a far wider application than that of coal mining alone: spark-proof equipment is vital in many industries. Eisner selected two at random: fork-lift trucks used in the chemical industry, and safe diesel engines which can be run in potentially dangerous atmospheres.



Discharge of water from disperser of triggered barrier.

Many government agencies are moving over to the system by which they choose at cost. The EFL does make charges at a commercial rate, although—as Eisner points out—the information provided is cheap at the price. Invaluable work has been done in the area of the testing of explosive devices for use in industry. Manufacturers of explosives have a difficult task: they are required to develop explosives which are safe for use underground, and which in many cases release an energy 10^8 times greater than that required for the ignition of any methane which may be present.

Some of the current research programmes at the EFL include computer model studies of uncontrolled vapour clouds and explosions and, following the recent grain silo accidents in the US, the explosive potential of these installations is receiving more urgent attention.

Although there has been no explosion involving coal dust in a British mine for the past fifteen years, there is no ground for complacency, according to Dr Donald Rae. Rae is one of the physcists at Buxton and is investigating automatic devices for the containment of explosions underground, and he maintains that there are occasional situations in which the stone-dust barriers in current use need to be replaced. The high rate of coal extraction in modern mining apparently makes automatic devices essential. Stone-dust barriers require a great deal of maintenance; Rae feels that the 'triggered barrier' could be the answer for the future. He and his colleagues have spent ten years developing this device, and the first will soon be installed in a British mine. At present stone-dust is tipped into the path of an explosion to 'snuff it out'. The triggered barrier uses water under high pressure to perform the same function. A thermocouple responds to the flame of the explosion, relaying the message to a pressurised nitrogen cylinder, the pressure of which is used to eject water from a second cylinder into the path of the oncoming flame.

The possible range of flame speeds

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underground is anything between 0 4 and 3,000 m s^{-1} (detonation speed). In practice, most explosions in their early stages travel at a speed of between 40 and 400 m s^{-1}, and the barrier has been developed accordingly and will ensure that sufficient water is available to extinguish anything in this range

The NCB was swift to realise the ignition hazard posed by light metals, although mining industries in some countries have still not taken action to reduce, or indeed eliminate, the hazard—some mines in the US are known to fall into this category. Frank Powell and Roy Gregory, both working at Buxton, demonstrated all too easily how a 'spark proof' tool with a handle of aluminium, or other light metal, will produce showers of sparks if struck on a rusty steel bar. With their high affinity for oxygen, these metals, when so struck, cause a thermite reaction, generating temperatures of about 3000 °C; the sparks which are simply the visible hot fragments of reacting metals may have sufficient energy to ignite methane, and would present an ignition risk if the metals were allowed into a mine. It is with some justification therefore, that Powell and Gregory tend to be rather dismissive about the safety qualities of some 'spark proof' tools And they add that it is not only the mining industry which has been shown to be at risk: for light metal anti-corrosion electrodes have caused several explosions in oil tankers.

Safety engineering

The Inquiry into the explosion at the Flixborough Chemical plant in 1974 concluded that poorly constructed and insufficiently supported by-pass piping at the 'Nypro' plant collapsed, allowing cyclohexane vapour to escape. Ignition of the vapour produced an explosion which was responsible for the death of 28 people. The Safety Engineering Laboratory (SEL) at Sheffield played its part in the investigation The work at Sheffield included forensic studies of the failed components and materials, involved as well as tests on a simulated by-pass pipe assembly

Entering the Incidents Laboratory at SEL is rather like visiting the yard of a scrap metal dealer. lifting gear, process-plant components, mining equipment and wire ropes are strewn over the floor of the lab Jack Deakin, an engineer in the laboratory, says that most of the items were removed from the site of accidents and either had been, or were about to be, examined by the laboratory The investigations done cover fatigue and brittle fractures, defective welding, and design and maintenance problems.

But engineering is not the only concern of the Sheffield Laboratory For the past few years fatal accidents in the mining industry have remained at about 50 per annum, following a steady fall over the previous 20 years as conditions in the mines improved. Indeed, the fatal accident rate 100,000 manshifts worked was almost halved between 1958 and 1971, but since then there has been no significant improvement And the fall in fatalities has not been matched by a decline in the serious injury rate.

Dr Clive Nussey (of SEL) believes that studies of minor accidents and 'near miss' situations might well provide information to help reduce the fatal and serious injury rate. (The chemical industry has recognised for a long time that the lessons to be learned from 'near misses' are as valuable as actual accidents, particularly as the former occur so much more frequently than the latter) Nussey says that over a third of the fatal and serious injuries in the mining industry are attributable to haulage and transport And in a collaborative study on minor injuries associated with transport and handling accidents, done with the NCB and HSE's Mines Inspectorate, Nussey's laboratory found that the events leading to these injuries, or indeed to 'near misses', also appear as causes of fatal or serious injuries on transport systems. Nussey believes, therefore, that even outside the mines the frequency of these events is such that they could form the basis of early warning system to help predict where more serious injuries might occur in future

Medicine and hygiene

The Occupational Medicine and Hygiene Laboratory is the third of the HSL Laboratories and is mainly located at Cricklewood The biological monitoring work in this laboratory is under the direction of Dr David Gompertz, he is relatively new to the post, and is still in the process of developing the laboratory facilities. He admits to being "aggressive and demanding" and to being impatient to change the emphasis of the work in his laboratory. When Gompertz moved in, 85% of the work of the Occupational Medicine Laboratory was on metals and only 15% on organic chemicals, and 95% of the time was spent doing routine investigations This will change

It is envisioned that in the future only 40% of the work will deal with metals, and 60% will be concerned with organic chemicals—but this does not mean there will be less work on metals. The routine investigations will still consume most of the time—about 60%—but Gompertz says that the rest will be devoted to research and development Gompertz is receiving support from the HSE for his programme of change. Evidence of that support is provided by one of the laboratories latest acquisitions, a gas chromatograph and mass spectrometer which will be used to build up a background library of chemicals for use in the biological monitoring of workers at risk.

The Occupational Hygiene Laboratory (OHL), also at Cricklewood, is much concerned with the measurement of contaminants—such as fibrogenic materials, toxic metals, gases and vapours—in the working environment. Work at the OHL centres on developing the methodology and instruments for use in such situations One of the instruments developed there, a general-purpose tape for monitoring atmospheric chemicals, is listed in the HSE's 1977 Research Report The few tapes currently commercially available are designed to have a long shelf life and to remain stable In consequence, they can only be used for a limited range of chemicals The HSE research team has now developed a more versatile tape with a wider application Although the report devotes no more space to this development than it does to other topics, this in no way lessens the impact for occupational hygienists The institute is also about to publish details of a tape for detecting aromatic amines; and hopes to produce ones for aromatic isocyanates, phosgene and chlorine

The technology for measuring respirable dust concentrations is constantly being improved and the OHL is placing much emphasis on another of its entries in the report, a continuously recording instrument for measuring dust samples. Called SIMSLIN II — the Safety in Mines Scattered Light Instrument—it is an improved version of an ealier model SIMSLIN II is currently at the pre-production stage and is being tested in underground trials in co-operation with the NCB SIMSLIN II weighs 8 2 kg and is 42 cm long and according to the report it is 'intrinsically safe in methane atmosphere'. The instrument is able to exclude non-respirable particles from the dust intake and to measure the respirable fraction by light scattering at regular—15 s or longer—intervals. With SIMSLIN II already being manufactured by a commercial company the OHL must be fairly certain of its marketability for it asserts in the report that "the instrument should find wide application in many industries where detailed records of dust concentrations are required"

The HSE appears to be acutely conscious of the need to produce useful research findings in the field of health and safety As it is, the 1977 report forms an impressive track record for a research year, and there are unlikely to be many complaints about the chosen allocation of the HSE budget ☐

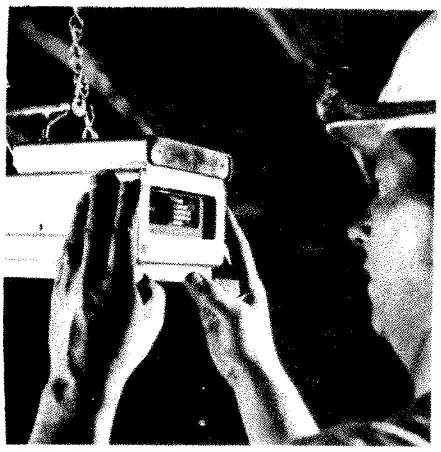
HSE's scientific mediator

On 1 July this year, Dr Ken Duncan became the third member of the triumvirate that runs the Health and Safety Executive (HSE). Duncan will be the principal person at HSE concerned with matters of biomedical research. He will combine his new work with his job of the past four years as Director of Medical Services on the staff of the HSE secretariat. Here he talks to ALASTAIR HAY.

OCCUPATIONAL medicine has been Ken Duncan's main concern for much of his working life, most of which has been spent in industry. His career began with British Rail and the South Western Gas Board. He later became chief medical officer of the United Kingdom Atomic Energy Authority and then head of health and safety at the British Steel Corporation.

Duncan has also played a part in the academic world. He is currently a visiting professor at the London School of Hygiene and Tropical Medicine, and registrar of the newly-formed Faculty of Occupational Medicine at the Royal College of Physicians. He is a former president of the Society of Occupational Medicine, has a place on the board of the Permanent Commission and International Association of Occupational Health, and is also a member of the Medical Research Council. It was on the subject of relations between the HSE and the MRC that he began his interview with *Nature*.

Duncan recalls that, when Rothschild's "transferred fund system" was implemented, the HSE found itself with some £700,000 to allocate annually to biomedical research. However, unlike the Department of Health and Social Security which was also affected by the Rothschild system, the HSE had few of its own research programmes in this field. It had to "buy itself into research," he says. "HSE's intention was to implement Rothschild by looking at specific problems. But there was one real difficulty. Very few customers had the necessary expertise to draft research protocols for specific problems." Fortunately, the MRC was on hand to help select suitable projects from the numerous proposals received.



Simslin recording the concentration of respirable airborne dust particles (see p.436).

The HSE will have to show that its £700,000 is well spent. If it fails to do so, one result, according to Duncan, will be a loss of trade union support, which could well be disastrous. The commission (HSC) is a tripartite organisation involving the Confederation of British Industry, local authority nominees and the Trades Union Congress. TUC support is obviously vital.

Duncan admits that the HSE places a heavy load on the MRC and that the council is not altogether happy with the arrangement. It is no secret that the MRC would prefer the HSE to follow the example of the DHSS on research work, but Duncan believes that the HSE has yet to reach this stage of development. Relations between the two bodies are reflected in the financial arrangements; until the HSE can assess its own research proposals, it will continue to ask the MRC for guidance, and while this is the case, the MRC has been reassured that most of HSE's research funds will be channelled through its books.

Under the terms of the Health and Safety at Work Act, the onus is on employers to prove the health and safety of each workplace. Duncan sees the HSE's role as monitoring health and safety projects carried out by industry, and cooperating with researchers "to ensure that the work is valid and in no way suspect".

He is deeply disturbed by the conflict in evidence in the United States between scientists in academic circles and those in industry. This polarisation has become so marked that in some cases neither group is prepared to accept the research results produced by the other. Academic scientists have voiced the opinion that the research work of industrial scientists in the field of toxicology is not only biased towards the companies involved, but that results have been deliberately kept secret and in some cases even destroyed. They also claim the research itself is often of poor quality. Scientists in industry have been swift in responding to these accusations. They say the charges may apply to a few companies, but not to the majority. Many have also been heard to complain that scientists in academic circles are making unrealistic and irresponsible demands upon industry for absolute safety.

Should this conflict spread to the UK, Duncan feels one of the most important functions of the HSE in the next five years could well be as mediator, by ensuring that industrial research, and in particular research into hazards, is of a reputable standard. He also indicates the importance of publishing all results even if they do not yield the "right answers". The health and safety act now makes it compulsory for employers to inform employees about risks (and for the manufacturer, installer and supplier to inform an industrial client similarly).

If the scientific integrity of the researcher could be accepted by all concerned, Duncan believes the conflict need not be so severe in Britain. He refers to the "constant dilemma" which many research workers face when only preliminary results are available. The question everyone asks, he says, is "do you publish the results or withhold the information until you have more evidence to confirm your observations?". Duncan is certain that industry would reveal more information if the response to it was not exaggerated. But he stresses that the one thing the HSE cannot do in this situation is to abet industry in 'covering up' important information. The response of a few trade unions to research on risks is still a crude one; they see it as a potential threat to jobs. Duncan is not enamoured of this response. Ultimately, however, risks will have to be evaluated rationally and this, he suggests, might be a job for the commission.

Duncan sees toxicology as one area in which the HSE will have to offer guidance to the commission for its deliberations. It is recognised that the new HSE notification schemes will generate some friction in industry, and toxicologists will be well to the fore in future controversies. The HSE's intention is to have "plenty of scientific kick" to back up its decisions, says Duncan. And the "kick" will come not only from the ranks of the HSE: the DHSS will be on hand to provide additional support. As a result of this arrangmeent, Duncan is confident that the executive will be well equipped to cope with the demands put upon it. □

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correspondence

Mincingly speaking

Sir,—My good friend Ken. Mellanby (7 September, page 8) fell into the trap of assuming that a word cannot have two meanings. Mincemeat is defined in dictionaries (including the Oxford Dictionary) as either (1) ground, chopped or minced meat, (2) the mixture of raisins, apples, spices etc used on both sides of the Atlantic in mince pies. The term 'mincemeat' for ground meat may have been abbreviated to 'mince' in Huntingdon—I do not know. But, as a former Sussex man, I recall that Hilaire Belloc said that the Midlands were "sodden and unkind". I would not think of using such language myself, of course.

Yours faithfully,
THOMAS H. JUKES

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mincemeat *n* [alter. of *minced meat*, fr. *minced* (past part. of ^{1}*mince*) + *meat*] 1: minced meat 2 : a finely chopped and usu. cooked mixture of raisins, apples, spices, and other ingredients with or without meat and suet 3 . something felt to resemble finely chopped meat; *specif* : a state of destruction or annihilation — used in the phrase *make mincemeat of* science of making .. of the old-time religion — F.L. Allen; making . of the inhabitants — Richard Joseph)

Geos III not enough for POLO

Sir,—Your articles on the European Space Agency (ESA) (5 October, page 355) are timely as its activities and those of its predecessor (ESRO) have not been widely known to the European scientific community: the present discussions within ESA of future programmes are greatly hampered by this ignorance. However, your statements about the polar orbiting lunar observatory (POLO) need clarifying. You mention the proposal to use the fourth Ariane test flight in 1980 to launch a lunar satellite, building this quickly and cheaply from Geos III hardware. However this is not the POLO concept as only a small part of the necessary complement of experiments to survey the whole surface of the Moon by chemical and physical sensors could be flown on this test flight.

Your report does not make clear that a mission definition study is now being undertaken so that the POLO can be a competitor in the decision as to which mission ESA will launch in 1985. In their recent report ('An approach to long range planning 1980-1990') the ESA Solar System Working Group laid great emphasis on the desirability of European involvement in studies of the Moon and planets and smaller bodies of the solar system, and it is as a result of the interest stimulated by this report that the mission definition study of POLO has been launched. This new development in ESA's thinking recognises that many European laboratories in the last decade have done fundamental work on Apollo and USSR lunar samples, and have participated in planetary missions (see 'The Moon: a new appraisal from laboratory studies and space missions'. The Royal Society. 1977. Ed. by G. Brown, G. Eglinton, S. K. Runcorn and H. C. Urey). The Ariane rocket has progressed so successfully that Europe will have in the 1980s the technology capable of launching orbiters for scientific study around Mars, the Moon and Venus.

During the preparatory study of POLO, it has been established that there are European laboratories capable of making the experimental hardware for all the various experiments which need to be done (see experiments listed in *Nature*, **265**, 197–199 (1977) Runcorn S. K. and Coleman P. J.) but there is now a great need for the European scientific community, particularly young physicists, chemists and geologists, to take these opportunities now unfolding in ESA, seriously. Potential interest from European scientists in building experiments or making use of the data to be obtained or perhaps suggesting entirely novel experiments to be done from the orbiter, which the rather closed circle of space scientists may not have envisaged, could be very helpful in the preparatory study now proceeding. (Dr G. Haskall, European Space Agency, 8-10 rue Mario Nikis, 75738 Paris Cedex 15 will supply information.)

Yours faithfully,
S. K. RUNCORN

Department of Physics,
University of Newcastle upon Tyne,
UK.

Chemi-osmosis and energised protons

Sir,—I write this letter to correct some impressions (2 November, page 8) left by your correspondent while describing the achievements of Dr Peter Mitchell for which the Nobel Prize was awarded. Mitchell's distinguished work in 1961 was in the development of a quantitative theory of chemi-osmosis and has been particularly used in explanation of the transduction of energy in biological systems. In this context chemi-osmosis uses a proton gradient. My contribution to this field, to which your correspondent refers most kindly, was to propose first that it was in fact energised protons which were the intermediates in energy transduction. The reference is *The Enzymes*, eds. P. D. Boyer, H. Lardy and K. Myrback, 1, 391; Acadamic Press 1959.

In 1961 I developed the idea of how energised protons might be used in systems which have diffusion control (*J. Theoret. Biol.* 1, 1; 1961) and one form of this control was independently visualised by Peter Mitchell and myself at that time. This is chemi-osmosis. Where Mitchell and I differed is that he considered this to be the actual mechanism in biological systems. Moreover he developed quantitative tests and did experiments to check his ideas. (It is also fair to add that chemi-osmosis unconnected with energy transduction and unknown to me was developed more qualitatively by Mitchell during the preceding five years. To be fair to myself, in 1961 before Mitchell's article in *Nature* **191**, 141; 1961 was written, he had read both of my papers above).

While Mitchell developed chemi-osmosis I developed an alternative more general approach to diffusion control based on my original concept of energised protons. I think you will understand that in this context I do not accept the concluding remarks by your correspondent about Popper's use of working words. It would be easy to show that Mitchell's ideas and mine could then become lost in the word "chemi-osmosis". Mitchell's theory must be examined in terms of the equations which were derived from it. As I do not accept that chemi-osmosis is a correct description of energy transduction in biology it is very important to keep names tightly associated with ideas. Clearly if in the long term my views are to predominate it is my job to show where chemi-osmosis is incorrect and where my own ideas of localised energised protons are correct. Some papers along these lines have been published and I am about to submit a detailed proposal.

The Nobel Prize has been awarded for chemi-osmosis. In that context it has been very properly awarded to Dr Peter Mitchell and I will be the last to contest this. It so happens that I do not think that it is the correct theory of the way in which energised protons (not originally asociated with chemi-osmosis, see above) are used in biological systems. We shall see.

Yours faithfully,
R. J. P. WILLIAMS

Inorganic Chemistry Laboratory,
University of Oxord, UK

US geneticists

Sir,—I wish to make two comments in connection with the article by John Douglas, 'US geneticists look to Europe for research facilities' (21 September, page 170):

- The list of sources of support of the original research leading to the procedure used in synthesising artificial genes should have included the American Cancer Society, not the National Cancer Institute.
- The penultimate paragraph cites the prospective United States patent as at least a partial reason why scientists might be doing certain genetic research in Europe.

The existence of a patent *per se* does not inhibit research in the US or elsewhere. The only basis on which this particular patent (if granted) might become a factor in the choosing of a site of research is that, as noted earlier in the article, industrial licensees of the patent will be required to follow the NIH safety guidelines for genetic-engineering.

Yours faithfully,
NIELS J. REIMERS

Office of Technology Licensing,
University of Stanford, USA

news and views

Interstellar carbon

from M. G. Edmunds

SOMETHING in interstellar space absorbs strongly at about 2200 Å. Since the discovery during rocket observations in the early 1960s of a broad absorption feature at this wavelength, more sustained access to the ultraviolet region of the spectrum by satellite observations has shown that the absorber is widespread in our own Galaxy. The detection of this 2200 Å feature in the Large Magellanic Cloud reported on page 478 of this issue of *Nature* by K. Nandy and D. M. Morgan indicates, perhaps not unexpectedly, that the absorber is a common constituent of the interstellar medium of galaxies. But the identification of the material responsible for the absorption remains in dispute. The considerable strength of the feature, normally observed by its influence on the light from a background star, implies that the absorber must be made up of one or more of the most abundant elements. Hydrogen and helium have no suitable features, and the next most abundant elements are oxygen and carbon.

Carbon in either solid or compound form seems to be the most likely candidate, and a similar absorption feature in the theoretical extinction properties of small graphite spheres was noted at the time of the first rocket observations. An elegant explanation of the exact shape of the observed feature was proposed by D. P. Gilra (*Scientific Results from OAO-2*, NASA SP-310, 1972), and this remains the most widely accepted evidence in support of the graphite sphere identification. He suggested that the absorption peak arises from the excitation of a collective mode of oscillation of the electrons, a so-called 'surface plasmon'. The match with observation is very good, provided the particles are very small (of order 0.01 μm in radius) and very spherical. Recent calculations of the expected wavelength dependence of extinction over both ultraviolet and optical spectral regions for a mix of particles of different sizes and compositions (Mathis *et. al. Astrophys. J.* **217**, 425; 1977) suggest that the exact size of the graphite spheres is not a very stringent limitation. The 2200 Å feature can still be reproduced by a distribution of graphite particles from 0.005 μm to .1 μm provided that the distribution is heavily weighted to smaller sizes. The fundamental restriction comes from the particle shape, because the wavelength and profile of the absorption feature will change significantly if the particles depart from almost perfect spheres. It may well be that the graphite component of interstellar dust is initially formed in a very spherical shape, or is perhaps subsequently modified to sphericity by collision or erosion. Yet the great anisotropy of the physical properties of graphite would lead to the expectation of a much less symmetrical linear or plate-like crystal growth. Good prospective sites for the formation of graphite dust are known—the carbon-rich atmospheres of some cool stars and the expanding, cooling ejecta of supernova explosions—but so little is known about the growth from vapour phase of small crystals under these low density conditions that no realistic predictions of grain shape can be made.

Other possible culprits for the 2200 Å feature have been proposed, and most of these again involve carbon. The detection at radio wavelengths of fairly large molecules has led F. Hoyle and N. C. Wickramasinghe to propose that a considerable fraction of interstellar carbon is in the form of complex organic molecules. The ultraviolet feature then arises by $\pi \rightarrow \pi^*$ electronic transitions in conjugated carbon bonds (*Nature* **261**, 241; 1976; **270**, 323; 1977). The organic molecules could be incorporated in or on dust particles, and the experimental detection of an absorption hump at 2200 Å in organic extract from the Murchison meteorite is evidence that a feature can be produced without requiring graphite spheres. The advantage of the bonding argument is that it removes the shape constraints on the dust which carries the molecules. Obviously detailed modelling is required to see if the shape of the observed feature can be accurately reproduced, although the authors admit that preliminary studies indicate that there is probably still a need for some contribution from graphite.

An interesting suggestion that the interstellar carbon may indeed be in solid elemental form, but as the allotrope carbyne rather than graphite, is currently being put forward by A. Webster of the Mullard Radio Astronomy Laboratory. This material (see for example, Whittaker, *Science* **200**, 763; 1978) is extremely difficult to make experimentally, and hence almost nothing is known about its physical properties. Its structure is basically of long parallel chains of conjugated carbon bonds, again giving rise to the hope that it might provide a 2200 Å feature by electronic transitions and independent of particle shape. Quantitative predictions of extinction properties will be required before the relevance of carbyne for the interstellar medium can be assessed. The (admittedly uncertain) phase diagram suggests that it might condense out before graphite in the proposed astrophysical sites of graphite formation, but it is not clear whether it would not subsequently convert to graphite on further cooling and exposure to radiation. Even if carbyne is only important as a short-lived stage in the condensation of graphite dust, this again has implications for the expected graphite dust shape, since the carbyne would probably grow as a linear structure and conversion to graphite is unlikely to produce perfect spheres.

The 2200 Å feature is not the only puzzle in the ultraviolet properties of interstellar material. The continuous extinction in the far ultraviolet considerably exceeds that which would be

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expected from the general extinction of the right amount of small graphite spheres required to match the 2200Å peak. The calculations on which this conclusion is based rest on classical Mie scattering theory, and there is some evidence (still unexplained) that experimental determinations of the ultraviolet behaviour of such small particles show a much higher extinction than the theoretical prediction (Day & Hufiman *Nature* **243**, 50; 1973).

It appears that for the present, despite the qualms about growth shapes, that small graphite spheres remain the best candidate for explaining the 2200 Å absorption. Yet work on alternative explanations is very important, particularly in the hope that an absorber can be found which not only accounts for this feature, but also for many of the broad shallow absorption features found in the optical spectral region and known as the 'diffuse interstellar bands' These have been observed for many years, but still defy convincing identification. There is no evidence that graphite spheres would supply such optical features, but on the grounds of simplicity it seems unsatisfactory to invoke yet more components of the interstellar medium to explain them, particularly as additional types of dust must already be proposed to account for the observed continuous extinction over the optical spectrum.

Two-neutron transfer heavy-ion reactions

from P. J. A. Buttle

THE growth in recent years of heavy ion physics, in which target nuclei are bombarded with nuclei accelerated in a heavy ion accelerator, has led to many interesting new phenomena in nuclear physics, but as is often the case with a new field of study, has introduced many new problems. Among the simplest types of reaction which can occur is the transfer of a single nucleon from one nucleus to the other Apart from a few anomalous cases, these single nucleon transfer reactions are reasonably well understood and it has been possible to obtain useful information about the wavefunctions describing the state of the transferred nucleon in the two nuclei. Reactions in which two nucleons are transferred have been more problematical, theoretical calculations often underestimating the yield of the reactions (or the cross section) by at least an order of magnitude.

Part at least of the difficulty is that the two nucleons can be transferred either simultaneously or in two separate steps (sequentially), whereas theoretical analyses have often been optimistically confined to only one of these mechanisms. To complicate matters further, the distinction between silmultaneous and sequential transfer is not very precise, so that the inclusion of both processes in a calculation involves a certain amount of double counting Full analysis shows that a 'non-orthogonality' term should be subtracted from the amplitude to allow for this.

A recent careful study of the two neutron transfer reaction

$$^{208}\mathrm{Pb} + {}^{16}\mathrm{O} \rightarrow {}^{206}\mathrm{Pb} + {}^{18}\mathrm{O}$$

at Minnesota (Franey *et al. Phys. Rev. Lett.* **41**, 837; 1978) confirms that both simultaneous and sequential transfer are important The experiments were done at a series of energies low enough (69–73 MeV) that the Coulomb repulsion between the charged nuclei prevents nuclear collisions The relative motion of the nuclei is then well understood and the transfer of nucleons from one to the other can be investigated without having to face the complexities of a full nuclear collision. The reaction is reasonably 'well matched', that is, the transfer of energy is fairly small so that the classical trajectories followed by the nuclei are not strongly perturbed by the transfer.

The single nucleon transfer reactions

$$^{208}\mathrm{Pb} + {}^{16}\mathrm{O} \rightarrow {}^{207}\mathrm{Pb} + {}^{17}\mathrm{O}$$
$$^{206}\mathrm{Pb} + {}^{18}\mathrm{O} \rightarrow {}^{207}\mathrm{Pb} + {}^{17}\mathrm{O}$$

to a series of excited states in ^{207}Pb and ^{17}O were also studied in order that both steps in the sequential transfer be properly understood

Two types of calculation were reported; the first a semi-classical calculation (Broglia & Winther *Phys Rept* **4C**, 153; 1972) in which the relative motion of the nuclei is treated classically. This is a reasonable approximation since the wavelength for classical motion is small compared with the size of the nuclei. Simultaneous, sequential and non-orthogonality terms contribute approximately 50%, 80% and −30% of the total amplitude, and the result is in excellent agreement with experiments

The second, fully quantal calculation (second order DWBA) gave a similar ratio for the simultaneous to sequential amplitudes. The non-orthogonality term could not be calculated and was assumed to contribute in the same proportion as in the semi-classical calculation. The overall magnitudes were however smaller, leading to an underestimate of the cross section. This may indicate that the non-orthogonality correction should be smaller in the quantal calculation, or that a more sophisticated treatment of the bound state wavefunctions is needed for simultaneous transfer, as suggested by Feng, Udagawa and Tamura (*Nuclear Phys.* **A274**, 262; 1976).

The general agreement between theory and experiment for two-neutron transfer is in accord with the analysis of Feng *et al.* for calcium targets, and gives more confidence in our understanding of these well matched reactions Difficulties remain, however, for less well matched reactions which have smaller yields, and for two-proton transfer reactions where theoretical calculations still underestimate experimental results by a large factor. ☐

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Pleiotypic effects of hormones

from Robert Shields

LONG before the dawn of molecular biology endocrinologists were aware that steroid hormones exert generalised trophic effects on their target tissues. For instance, oestrogens promote the growth and development of the uterus, glucocorticoids are trophic in the liver and androgens affect male accessory tissues such as the prostate and seminal vesicle. However, these effects are often conveniently forgotten by biologists attempting to understand the action of steroids at the molecular level Concentrating their efforts on the effects of steroids on a handful of specific proteins such as the egg white proteins of the chick oviduct, workers in this field have made impressive progress culminating in the successful cloning of the ovalbumin gene (Dugaiczyk *et al Nature* **274**, 328; 1978; Mandel *et al. Cell* **14**, 641, 1978) It will not be long before we understand how steroids act at the molecular level in at least a few restricted cases. The time has now come when the more general (and equally important) trophic effects of steroids should be re-examined

While the general trophic (or pleio-

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typic) effects of steroids may be the end product of a series of events involving the production of a few new mRNAs (and hence a few new proteins) it seems likely that additional mechanisms are involved. The view that the general trophic effects of steroids and their specific effects on a few proteins may have a different basis is reinforced by the observation that general and specific effects may become separated. For instance, hydrocortisone has a general anabolic effect on the liver and also promotes the production of a specific set of proteins, principally those involved in gluconeogenesis. In hepatoma cells, however, the trophic effect is absent, but the specific effect on the production of a few proteins including tyrosine transaminase is still present (Ivarie & O'Farrell *Cell* **13**, 41; 1978). Until recently there were few well-studied cases of the reverse situation where the general trophic effect of steroids could be observed without concomitant specific effects. Recent work on the rat seminal vesicle suggests that this may be a good system to do just that.

About 30% of the protein synthesised in the rat seminal vesicle is accounted for by two secreted glycoproteins of the seminal plasma. The synthesis of these proteins is controlled by testosterone since their synthetic rates fall after castration and rise after hormone treatment (Higgins *et al. Biochem. J.* **158**, 271; 1976). So far this looks just like another case of specific protein induction by steroid hormones Where these results are interesting is that the synthetic rates of these proteins follow the overall rate of protein synthesis which in turn parallels total mRNA levels (Higgins & Burchell *Biochem. J.* **174**, 543; 1978). So in this case the rise (or fall) of the levels of the mRNAs of these two proteins is not a consequence of the specific rise (or fall) of their respective mRNAs (as is the case with ovalbumin for instance) but is a result of some general change in metabolism of the entire spectrum of mRNAs in the tissue. Careful analysis of the sequence complexity of the mRNAs isolated from the tissue in different hormonal states confirms this conclusion (Higgins *et al. Eur. J. Biochem* in the press) and furthermore, these changes in total mRNA levels also parallel changes in the total RNA content of the organ (Higgins & Burchell *Biochem. J. op. cit.*). Since mRNA accounts for only a fraction of the total RNA in the cell this result shows that in this tissue at least, androgens may regulate the overall levels of RNA transcription rather than the transcription of a severely limited number of genes.

These results are strong evidence that androgens act to produce the trophic response in the seminal vesicle without inducing new mRNA species; however, in other tissues androgens are capable of inducing new proteins. In the ventral prostate, for instance, androgens control the synthesis of four major proteins that, like the seminal vesicle proteins, are glycoproteins secreted into the seminal plasma (Hayns *FEBS Lett* **81**, 43; 1977; Parker *et al. Biochem. J.* **170**, 115; 1978). In distinct contrast to the situation in the seminal vesicle these proteins are not regulated by general changes in overall mRNA synthesis but rather by specific effects on their individual genes (Parker & Mainwaring *Cell* **12**, 401; 1977; Hayns *et al. Biochem. biophys. Res. Commun.* **77**, 1492; 1977; Parker & Scrace *Eur. J. Biochem.* **85**, 399; 1978).

These results show that alterations in the synthesis of major secretory proteins may be brought about by two different means, one is the (dare one say) more traditional alteration in the levels of specific mRNAs coding for those proteins, and the other is through modulation of the production of RNA as a whole. Quite why these two different strategies are adopted for the same hormone in functionally closely related tissues of the same animal is however far from clear. ☐

Amorphous silicon

from a Correspondent

The letter published in this issue of *Nature* (page 482) by S. R Ovshinsky and A Madan highlights the intense activity in laboratories all over the world seeking to exploit the potentialities of amorphous silicon for photovoltaic solar cells. For a solar cell which does not make use of lenses or mirrors to concentrate the Sun's radiation, it is obviously necessary to cover a very large area, and the outstanding advantage of deposited films of amorphous silicon is their cheapness, compared with the single crystals of the usual silicon technology.

The possibility of using amorphous silicon is a very recent development. It is only 15 to 20 years since it was recognised that glasses and amorphous materials generally have conduction and valence bands very similar to those in crystals The early work of Kolomiets and coworkers in Leningrad on the semiconducting chalcogenide glasses, for instance As_2Te_3, showed that, unlike crystals, these glassy semiconductors cannot be doped. The reason seems to be that in a glass any atom takes up a configuration such that all its outer electrons form bonds with nearest neighbours. Thus As has three neighbours in the glass, Te two and if Ge is added we expect it to have four Amorphous silicon is not of course a glass, in the sense that it cannot be prepared from the melt; but films prepared by deposition have the normal fourfold coordinations and behave as semiconductors. Properties of evaporated or sputtered films are not the same as those that would be expected from a continuous random network, in which every atom is bonded to four neighbours. For instance, electron paramagnetic resonance investigations reveal a high concentration of unpaired spins, presumably located at dangling bonds occurring on silicon atoms bonded to only three neighbours. Moreover these and other places where the continuous random network is broken produce what are called 'deep states in the gap', that is to say points where an electron may be trapped with a value of its energy somewhere midway between the conduction and valence bands. Such states, as also in crystalline semiconductors, act as recombination centres; if an electron and the corresponding positive hole are formed by the absorption of radiation, they recombine much faster in the neighbourhood of one of these deep states, which allows the electron to get rid of its energy in two steps. In fact, so rapid is the recombination in evaporated films that it is difficult to observe a photocurrent.

The Dundee school under W. Spear, which included at that time the junior author of the paper under discussion, showed that in films prepared by the deposition of silane (SiH_4) from a flow discharge, the number of these deep states could be very much reduced, so that a significant photocurrent could be observed. There has been some dispute about why this is so but it now seems reasonably certain that, instead of dangling bonds, Si-H bonds are formed, which do not give rise to states in the gap. The films appear to contain 5% of hydrogen or more and if the hydrogen is introduced in other ways the properties are similar. Some deep states, however, remain; there has been much speculation as to their nature, and also much effort to reduce their number, so as to reduce the rate of recombination.

Now the paper by Ovshinsky and Madan claims that fluorine will do all that hydrogen did and more; it reduces the number of states in the gap considerably more than hydrogen does, and gives greater stability. The importance of this lies in the discovery by the Dundee group a few years ago that, unlike Kolomiets' glasses, one can dope hydrogenated amorphous silicon, for instance by adding phosgene to the silane Some, but not all, of the phosphorus appears to go in with fourfold coordination, and this produces donors.

It has not yet proved possible to incorporate a concentration of donors greater than the concentrations of states in the gap, and therefore the donors lose all their electrons to these states. But the Fermi energy is thereby raised and can be brought quite near to the bottom of the conduction band. And similarly, by doping with boron, it can be brought near to the edge of the valence band. It is thus possible to produce a p–n junction, or a Schottky barrier, and thus a photovoltaic cell.

From these results from Ovshinsky's laboratory, it seems then that this can be done with less doping and greater stability by replacing hydrogen by fluorine. This may turn out to be quite an important step on the long road to an efficient solar cell. □

Vertebrate skin

from R. I. C. Spearman

THE successful investigation of any organ system involves many different disciplines. This was especially evident at a recent symposium on the 'Skin of Vertebrates'* held recently in London.

R. J. Roberts (University of Stirling) and A. Bullock (Scottish Marine Biological Association, Oban) have found that in teleost fish the epidermal cells are mitotically active at all levels. This is in contrast to mammals, in which cell division is normally limited to the basal layer. In consequence, skin wound healing in fish occurs without the need for a preliminary burst of mitotic activity as seen in mammalian epidermis.

A. M. Lucas (Michigan State University) and A. G. Matoltsy (State University, Boston) showed that chicken epidermis is peculiar in that appreciable free lipids are endosecreted into the cytoplasm in addition to keratin. In contrast, mammalian epidermal cells produce little free lipid except in the sebaceous glands. R. Wrench (University College Hospital Medical School, London) has found extensive cytolysis in chicken epidermis as in sebaceous glands and she suggested that chicken epidermis is intermediate in type between the keratin-forming mammalian epidermis and lipid-secreting skin glands.

Studies on the supramolecular structure of the hair keratin complex which is constructed from many different proteins have made major advances in the past few years with long amino-acid sequences now known for some of the constituent proteins. For many years it had been thought that disulphide linkages stabilise keratins by forming cross-links between protein chains. However, R. D. B. Fraser (CSIRO, Melbourne) pointed out that there now seem to be very few disulphide cross linkages and most disulphide bonds link different points on the same chain. Nevertheless, reducing agents which break these intra-chain bonds, such as depilatory agents, soften hair keratins. How such intrachain linkages can stabilise arrangements between adjacent protein chains is not clear, but is of importance in the bonding of high molecular substances in general.

Since the turn of the century it has been known that the nerves to a tissue have some stimulatory effect on cell growth. This was first noticed in cases of gunshot wounds in which the severance of nerves to the hand had slowed down the rate of nail growth. The work of M. Singer (Case Western University, Ohio) and his colleagues on the action of nerves on limb regeneration in amphibia showed that both motor and sensory neurones promote epidermal cell division, RNA transcription and protein synthesis. The active agent has been found to be a protein which has been isolated from nerves.

Both fetal skin differentiation and adult modulation depend on interactions between dermis and epidermis. This has been studied using recombinants of dermis and epidermis from different sites. P. F. Goetinck (Connecticut State University) has found that some genes for keratinised structures act on the dermal cell transmitter of the inductor stimulus and others on the epidermal cell receiver. P. Sengel (University of Grenoble) showed that in embryonic life both the regional epidermal morphology and the particular keratin proteins formed by epidermal cells are dependent on induction by dermal cells. This is even true of recombinants between fetal mouse epidermis and chicken dermis, and so the ability of dermal cells to control epidermal functions can be transmitted across zoological class barriers. The epidermis retains some intrinsic rhythmic activity as in the sloughing cycle of snakes and lizards.

The work of A. W. Poole (Rutgers University, New Jersey) also using tissue recombinant methods has shown numerous interactions between melanocytes and epidermal cells of the hair follicle. Thus, some mutant genes which alter coat coloration act directly through the melanocytes and others act on the hair formative cells to modify the melanin pigmentation. D. T. Parkin (University of Nottingham) studied pigmentation polymorphism from another viewpoint; the effects of ecology and sexual selection on population genetics. A good example of this is the changing pigmentary patterns of pigeons seen in city streets.

As A. Iggo (Royal Dick School of Veterinary Studies, Edinburgh) pointed out in a final session devoted to neural sensation, to understand the functioning of a sensory receptor it is necessary to study the electrical activity of an efferent nerve in response to different specific stimuli, which must then be correlated with histological findings. There is good evidence that the mammalian Pacinian corpuscle and the Meissner corpuscle are pressure and touch receptors respectively, and the fact that separate sensory areas in the brain are present for heat and cold would indicate special receptors for thermal senses, although the physiological work on these is incomplete. The suggestion put forward some 20 years ago that there are no specific sensory receptors has prompted extensive research on the subject by those who believe in specificity. However, various speakers showed that in all vertebrate classes microscopy of sensory receptors has greatly outstripped physiology, and it will be some years before we fully understand skin sensation, even in mammals. □

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*Held on 25–27 September and organised by the Linnean Society of London in conjunction with the British Association of Dermatologists. The Proceedings will be published for the Linnean Society by Academic Press.

Accuracy in the transmission of biological information

from a Correspondent

THE 'recognition' of one biological molecule by another has long been acknowledged to be one of the fundamental physical principles of biology. In all cases the recognition is a first step, preceding a subsequent reaction in which the correct substrate reacts further along a path from which incorrect substrates must be excluded.

More recently it has become possible to describe in quantitative terms the correctness of the recognition using an error level parameter, which expresses the ratio of the numbers of correct and incorrect substrates reacting. The error level can in many cases be measured, for example by offering *in vitro* the correct and incorrect substrate in equal amounts and examining the formation of correct and incorrect products. Typical error levels are, for protein synthesis from mRNA, 1 in 10^4 (one

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*EMBO Workshop, 'Accuracy', was held in Grignon, France, on 12–15 September, 1978 and organised by Dr Jacques Ninio.

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wrong amino acid incorporated for every 10^4 right ones) and for DNA replication 1 in 3×10^4. *In vivo* the error level in protein synthesis can be measured by observing a mutant in which the gene for a particular enzyme is blocked by a nonsense mutation; the appearance of small amounts of this enzyme in spite of the mutation gives a measure of the rate at which the nonsense is misread as sense.

Error levels found *in vivo* are often lower than those found *in vitro* which means that (neglecting artefacts of extraction) the organism can and does reduce its error level by increasing the complexity of the recognition process —spontaneous mutation rates fall in the range of 1 wrong base for every 10^7 to 10^{11} right ones. The error levels expected can also be predicted thermodynamically by appeal to the relationship between order and entropy: the selection of a correct substrate rather than a random one involves an increase in the amount of order in the biological system and is therefore thermodynamically unfavourable, unless other factors can be brought into play. These factors are, in part, well known: the Watson-Crick interaction in DNA replication, surface complementarity between enzyme and substrate, codon-anticodon binding on the ribosome. Since the energy involved in the selection is often known (for example, the difference between the energies of formation of a correctly-matched and a mismatched Watson-Crick pair) the error level can be predicted using simple thermodynamics. Such predicted error levels are very frequently orders of magnitude higher (that is, worse) than those achieved in cells, demonstrating yet another feat of nature which chemists can at present neither reproduce nor properly explain.

In the hope that this problem might, however, yield one day to the combined onslaught of interested mathematicians, physicists, chemists, biochemists and biologists, a meeting on 'Accuracy' was held recently*. Of principal interest were problems such as those outlined above—DNA polymerisation, protein synthesis, codon recognition, information and its relation to thermodynamics and kinetics —but the meeting also included related topics such as antibody specificity, accuracy and evolution, protein degradation and ageing.

The greatest interest was aroused by subjects relating to the central problem of molecular biology—the transmission of hereditary information over many generations of DNA and the realisation of this information in the synthesis of protein. To explain the low error levels seen in these processes, the idea of 'proofreading', has been postulated by Ninio and Hopfield (Ninio *J. molec. Biol.* **84,** 297; 1974; Hopfield *Proc. natn. Acad. Sci. U.S.A.* **71,**; 4135; 1974). They have suggested that in cases where a low error level is important the bound substrate is double-checked in a second recognition step, and rejected if it is the wrong one.

Proofreading may take place in many ways, so that according to C. Kurland (Uppsala) "we now have more theories to explain accuracy in protein synthesis than experimental results to test them." However, the proofreading theory does make falsifiable predictions, one of which is that the offer of a great excess of wrong substrate should result in a high turnover of the first recognition step, with rejection of the wrong product at the second. This has been observed by R. Thompson (University of Pennsylvania, Philadelphia) who compared the amounts of energy-rich triphosphate consumed when an artificial mRNA was used in the synthesis of the correct or (under forcing conditions) the incorrect polypeptide. For every incorrect amino acid incorporated around 30 triphosphate molecules were hydrolysed, implying that this number of incorrect amino acid molecules is eliminated at the second recognition (proofreading) step. The incorporation of the correct amino acid, which is not rejected at the proofreading step, proceeds of course much more rapidly and less wastefully.

Other forms of proofreading postulated include one by F. von der Haar and F. Cramer (University of Göttingen), in which the chemical environment of the recognition site enhances the accuracy of the recognition ('chemical proofreading'). In

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Bronze Age honey

from Peter D. Moore

THE analysis of pollen grains from samples of honey is now a commonly used technique for determining what plants are used by foraging bees. It has recently been put to a rather unexpected use by Dickson (*Antiquity* **52,** 108; 1978) in the analysis of materials from a Scottish Bronze Age barrow at Ashgrove, Fife.

Within a burial cist a large quantity of decomposed plant material was found, containing the fruits of birch and seeds of rushes, together with mosses including *Sphagnum*. Over the skeleton there was a deposit of black crumbly material which, when subjected to analysis, was found to contain pollen in the proportions 54% lime (*Tilia*), 15% meadowsweet (*Filipendula*), 8% heather (*Calluna*) and 7% plantain (*Plantago lanceolata*) as well as various other minor components. The very high values for lime are exceptional, for this tree is insect pollinated. It is therefore unlikely that this assemblage represents the contemporaneous pollen fallout when the burial took place.

Dickson considers two possible explanations. It is conceivable that flowers were placed within the tomb and, since lime and meadowsweet both flower in July, their presence together is reasonable. Glob (*The Mound People*, 61, Faber and Faber, 1970) describes the use of flowers in the Bronze Age burials of Denmark. Yarrow (*Achillea millefolium*) was found in an oak coffin and this plant was used extensively both for foretelling the future and for healing wounds. No such significance is attached to lime or meadowsweet.

An alternative explanation, which Dickson favours, is that an overturned beaker also found in the burial may have contained honey or mead which had originally been produced from lime flowers. This would also explain the presence of meadowsweet (medesweete) which was widely used as an additive to mead. Dickson has found support for his argument in the analysis of scrapings from the outside and inside surfaces of the beaker. Only the inside contained lime and meadowsweet pollen, the greatest concentration being upon the lower surface of the tipped vessel. It therefore seems certain that the beaker contained honey or a honey derivative, making it the oldest record of honey in Britain, with a radiocarbon date of 3046 ± 150 b.p.

Dickson is inclined to believe that the honey was imported either from England, where lime was a far commoner tree in Bronze Age times (judging from the level of its pollen in peats and lake sediments) or even from Denmark, where Bronze Age honey has previously been recorded. There is, however, no firm evidence upon which to come to a conclusion. A single lime tree in the vicinity of a hive could account for the pollen assemblage, and lime would be capable of flowering in Fife even if it could not set ripe fruit. So the possibility still remains that this first Scottish honey was home produced.

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another, postulated by A. R. Fersht (Imperial College, London) the amino acylation site rejects competing amino acids which are larger than the correct substrate but accepts smaller amino acids. These pass to a second, hydrolytic site which, again on steric grounds, rejects the correct substrate but destroys the reaction product formed with the competing smaller amino acids.

This mechanism has been dubbed 'double sieve editing'. R. Mulvey (MRC Laboratory of Molecular Biology, Cambridge) presented results which indicate that this is how, for example, valyl-tRNA synthetase rejects with such efficiency competitors which differ merely by one methylene group from the correct substrate, valine.

If the occurrence of proofreading can be finally confirmed, then future work in this field will clearly include the search for non-proofreading mutants and for a molecular description of the proofreading process itself, which, in spite of its complexity, must have originated at a very early stage in the evolution of the cell. □

Carcinogen binding to DNA

from Stephen Neidle

It is now generally acknowledged that the polycyclic aromatic hydrocarbons are not directly carcinogenic but are metabolised *in vivo* to short-lived reactive species that bind to cellular macromolecules, initiating cancerous processes in some way. Most evidence indicates that DNA is the critical receptor—a conclusion strengthened by the relationship between mutagenicity and carcinogenicity indicated by 'Ames-type' bacterial test systems. Over the past few years much activity has been directed at understanding the chemistry of the interactions of such metabolites with DNA But why such events are carcinogenic remains unknown.

In the case of benzo[*a*]pyrene, an important metabolite seems to be the *trans* isomer of its 7,8-diol,9,10-epoxide derivative; this electrophilic compound has far greater mutagenic and carcinogenic activity than other isomers It binds predominantly to guanine residues in DNA, with the N2 position of the purine base being thought to be a major site of attack. (However, this picture is complicated by the recent finding of Osborne, Harvey & Brookes (*Chem.-Biol Interactions* **20**, 123, 1978) that the N7 atom of guanine is also susceptible to attack by the diol epoxide; the instability of this adduct explains why it was not detected in previous studies).

Identification of likely sites on DNA for covalent attachment of a carcinogenic molecule is a vital prerequisite for any attempt at visualisation of the conformational and structural changes induced in the DNA. Grunberger, Weinstein *et al.* have utilised such information in an examination (by means of circular dichroism spectroscopy) of the conformational properties of the dinucleoside phosphate GpU to which was bound benzo[*a*]pyrene diol epoxide, at the N2 position of the guanine base (*Biochemistry* **17**, 1278; 1978) GpU was considered to be a model for a nucleic acid; however this can only be true in a restricted sense since it cannot base-pair and thus form a fragment of double-stranded polymer. Nevertheless, the finding that the bound polycyclic hydrocarbon interacts in some (probably stacking interaction) manner with the uracil residue, is of interest and some significance As Grunberger *et al.* point out, such an interaction profoundly alters the conformation of the molecule around the guanine–sugar linkage, such that if it were in a double strand the guanine would be forced out of its base-pairing position Grunberger, Weinstein *et al* have previously shown (*Biochemistry* **16**, 3127; 1977) that benzo[*a*]pyrene diol expoxide–modified DNA has small local regions of denaturation and instability, as instanced by increased sensitivity to S_1 nuclease attack relative to native DNA.

These molecular concepts have now been taken a step further by Drinkwater, Miller and Yang (*Cancer Res* **38**, 3247, 1978) who have examined the binding and mutagenic behaviour of a number of aromatic electrophilic carcinogens. These ranged from the potent benzo[*a*]pyrene diol epoxide to simple ethylene oxide–aromatic derivatives. Reasoning that the planar aromatic groups common to such molecules could well intercalate into DNA (an old, but hitherto largely discredited theory), Drinkwater *et al.* measured the unwinding induced in closed circular supercoiled SV40 DNA. This technique is widely used as a tool in the investigation of known intercalative drugs such as ethidium bromide, proflavine and actinomycin Unwinding and consequent reversal of supercoiling is considered to be diagnostic for intercalative binding Such an effect was observed for almost all the compounds studied, although with striking differences in unwinding activity between the diol epoxide and 1-pyrenyloxirane on the one hand, and such compounds as 7-benzanthryloxirane on the other. Interestingly, a strong positive correlation was found between unwinding ability, and frame-shift mutagenic activity (in a *Salmonella* Ames test) for the range of compounds tested Overall, the results of Drinkwater *et al.* clearly point to local unwinding of DNA being the consequence of the covalent binding of an active compound. They have used this information as the basis for a plausible molecular model for the structure of the DNA adduct In agreement with the notion of local denaturation at the binding site, the residue to which for example, benzo[*a*]pyrene diol epoxide is bound (be it at the N2,N7 or even C8 positions), becomes destacked from its adjacent bases and thus protrudes to some extent The aromatic part of the carcinogen is postulated to intercalate between the adjacent bases, in a similar way to the non-covalently-bound intercalating drugs. Confirmation of this model in detail must await a definitive X-ray structure analysis of, say, a carcinogen–deoxyoligonucleotide complex. The visualisation of drug–nucleic acid binding in this way is currently a topic of lively interest; it is to be hoped however that in the case of covalently-bound carcinogens, the pitfalls of extrapolating from relatively simple model complexes to polymers, will be adequately perceived

That guanine residues are the major target in DNA for diol epoxide attack, is now well established; however what is rather less clear is whether such bindings themselves are the major carcinogenic events, or indeed what relationship they have to such events The complexity of the situation is emphasised by the recent work of Kakefuda and Yamamoto (*Proc natn Acad Sci U.S A* **75**, 415; 1978) who have also examined benzo[*a*]pyrene diol epoxide modification of DNA They, like Drinkwater *et al.* observed local unwinding of closed circular DNA. However, analysis of the S_1 nuclease-treated product showed that predominantly adenine, and not guanine residues, were modified so as to cause local unwinding-induced denaturation, even though the latter bases were the major sites for actual binding of carcinogen. Furthermore, examination of double-stranded synthetic polynucleotide–carcinogen complexes showed that only the A-T one was S_1 sensitive Kakefuda and Yamamoto thus suggest that there are (at least) two categories of base modification, adenine involvement producing local unwinding and base-pair cleavage, and guanine attachment, with an altogether less drastic resultant However, the general features of the molecular model proposed by Drink-

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Stephen Neidle is in the Department of Biophysics, King's College London water, *et al.*, are unaffected by any such controversies concerning the precise nature of the carcinogenic binding site, and so may well be found to be roughly correct. ☐

The sequence of *E. coli* 16S ribosomal RNA

from Richard Brimacombe

THE development of rapid gel techniques for sequencing nucleic acids has provoked a scramble to finalise the sequences of the *E. coli* ribosomal RNA molecules. Several research groups have taken part in this race, and two complete sequences of the 16S RNA have just been published. Pride of place goes to the Californian group (Brosius, Palmer, Kennedy & Noller, *Proc. natn. Acad. Sci. U.S.A.* **75**, 4801; 1978) whose sequence was determined from a plasmid DNA containing one of the 16S ribosomal RNA genes, using the DNA techniques of Sanger and Coulson (*J. molec. Biol.* **94**, 441; 1975) and of Maxam and Gilbert (*Proc. natn. Acad. Sci. U.S.A.* **74**, 560; 1977). This sequence was first reported at the EMBO ribosome workshop in Salamanca in June (see *News and Views* **247**, 743; 1978), and it has been closely followed into print by a complete sequence from the Strasbourg group who presented a partial sequence based on gel data in Salamanca, which has since been finalised (Carbon, Ehresmann, Ehresmann & Ebel, *FEBS Lett.* **94**, 152; 1978).

This latter sequence, obtained entirely independently and without so much as a mention of the Californian group, is derived from a direct analysis of RNA-fragments, using the technique first applied to a polyribonucleotide by Donis-Keller *et al.* (*Nucleic Acids Res.* **4**, 2527 (1977)).

Publication of these two sequences closes a subject which has been a matter of controversy for some years. Work on the 16S sequence using classical techniques was initiated by the Strasbourg group about 10 years ago, and led, after some preliminary papers, to the publication of a detailed oligonucleotide catalogue (Fellner *et al. Biochimie* **54**, 853; 1972) and to a first tentative sequence of the molecule (Ehresmann *et al.* *Biochimie* **54**, 901; 1972). However, two years later, a paper appeared from Woese's group in Illinois (Uchida *et al. J. molec. Evol.* **3**, 63; 1974) which claimed that over 50% of the ribonuclease T_1 sequences published by the Strasbourg group for oligonucleotides of eight or more bases in length were incorrect, and this was shortly followed by a second confirmatory paper by the same group (Magrum *et al. Nature* **257**, 423; 1975). At about this time the Strasbourgers went to press with a new version of the 16S sequence (Ehresmann *et al. Biochimie* **57**, 711; 1975). This sequence, which claimed to be 90–95% complete, was very considerably modified with respect to the 1972 version, and some but by no means all of the T_1-oligonucleotide sequences were altered to agree with those of Woese's group. At this stage about 25% of the T_1-oligonucleotides remained to be finally ordered within the sequence, but there were also queries being raised concerning some of the existing orderings (see for example Noller *Biochemistry* **13**, 4694; 1974).

The next version of the Strasbourg sequence appeared in 1977 (Ehresmann *et al. FEBS Lett.* **84**, 1977), and contained a number of substantial improvements. Additional information was given for nucleotides in regions totalling over 300 bases, the number of T_1-oligonucleotides remaining not finally ordered was reduced to a total of just over 200 bases, and almost all of the T_1-oligonucleotide sequences were in agreement with Woese's data. At this stage, recognising that application of the classical methodology was becoming subject to a law of rapidly diminishing returns, the Strasbourg group turned their attention to gel sequencing technology, and have now completed the sequence as quoted above. In this final version, all the T_1-oligonucleotides are ordered and in addition there are alterations to sequences involving a total of almost 200 bases with respect of the 1977 data, which of course brings us to the crucial question—how well does this latest RNA sequence agree with the DNA sequence obtained by Noller's group? And the answer is, the agreement is excellent. The Californian sequence is 1,541 bases long, the Strasbourg sequence 1,542 bases, and within this whole length there are (by my count) only eight single-base discrepancies. The data agree also with sequences at the 3′ and 5′ ends of the molecule obtained by Young and Steitz (*Proc. natn. Acad. Sci. U.S.A.* **75**, 3593; 1978), and all of the Woese T_1-oligonucleotide sequences (with one possible exception, containing one of the eight above-mentioned disputed bases) are correct. So everybody should be happy, and the way is now clear for serious attempts to be made to determine the secondary and tertiary structure of the molecule.

It is perhaps worthwhile to consider very briefly here the relative merits of the DNA compared with the RNA gel methodology for the sequencing of long RNA molecules. Aside from the purely technical viewpoint (where any comparison of the two sets of sequencing data is difficult, since the Strasbourg publication contains no experimental documentation), there are several points to consider. First, the DNA method gives the sequence of a single cistron, whereas the RNA method gives the 'average' of all the cistrons for the RNA molecule concerned; this undoubtedly accounts for the small but significant number of heterogeneities observed in the 16S RNA by the Strasbourg group. Second, the DNA method has an obvious advantage in allowing the use of restriction enzymes to generate the fragments for sequencing; in the RNA method, the production of suitable fragments is a rather hit-and-miss affair, although here it is noteworthy that the Strasbourg group have for this purpose made good use of the double-strand-specific cobra venom nucelase of Vassilenko and Ryte (*Biokhimia* **40**, 578; 1975). Third, while the DNA method is for this last reason certainly the cleanest and quickest for sequencing the whole molecule (once the appropriate cistron has ben isolated), the RNA method is likely to prove most useful for people working with ribosomes or similar particles and wanting a rapid analysis of a particular region of the structure. Finally, it should not be forgotten that the classical methods of oligonucleotide analysis are still needed, both to establish the nature and location of any post-transcriptional modifications such as methylation, and—particularly in the case of the RNA gel method, as has been clearly shown in a similar sequencing study by Gross *et al.* (*Nature* **273**, 203; 1978)—to confirm the sequence. ☐

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Meteorology over the tropical oceans

from D. B. Shaw

A CONFERENCE on meteorology over the tropical oceans was held in London during August this year.* By its very nature, meteorology is a science requiring international cooperation and it was fitting that the conference was jointly arranged by the Royal

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*The proceedings of the conference, in which the current state of the science is reviewed by ten leading authorities in their fields, are to be published early in 1979 by the Royal Meteorological Society, James Glaisher House, Grenville Place, Bracknell, Berkshire

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Meteorological Society, the American Meteorological Society, the Deutsche Meteorologische Gesellschaft and the Royal Society. One of the main purposes of the conference was to provide a platform for the presentation of research related to GATE, the GARP (Global Atmospheric Research Programme) Atlantic Tropical Experiment. The field phase of GATE was completed in 1974, and has been described by Mason (*Nature* **255**, 17; 1975). Since then various designated data centres have been processing the data to make them available to the meteorological community at large. Much of that work is now completed and many research projects making use of the data are now underway. The conference provided an opportunity to assess the progress that has been made in tropical meteorology in the past few years and to identify the outstanding problems.

Central to the purpose of GATE was the problem of scale interaction—the way in which convection, which is a vital heat transfer process in low latitudes, acts on and reacts to the larger scale flows of the tropical atmosphere. A fundamental obstacle in understanding this interaction is the relatively small scale of convective processes. Deep penetrative convective towers occupy only a tiny fraction (about 1%) of the tropical atmosphere. The standard observational network cannot hope to give an adequate account of such small scales of motion. They also present a difficulty to the numerical modeller. Most numerical models of the atmosphere have a spatial resolution too coarse to resolve convective processes explicitly. Their effects are simulated, or parameterised, by idealised formulations which are designed to represent the effect, not of an individual convective cell, but of the hypothetical ensemble of cells acting in the region represented by each of the model's grid points.

As a result of GATE the meteorologist now has, for the first time, a fairly detailed description of the atmosphere on scales from the synoptic down to the convective, for part of the tropics at least. The three-dimensional structure of the large scale (~2500 km) travelling disturbances over West Africa and the East Atlantic has now been well documented. These easterly waves, usually found in the months from June to October, have associated with them marked convective activity which is generally found in the region ahead and slightly to the south of the vorticity centre of the disturbance. From a consideration of energy exchange processes it can be deduced that the latent heat release in the convection plays an important part in the development of the wave over West Africa. The role of convection later in the life cycle of the disturbance (that is, over the ocean) is less clear. Of course the convective mechanism is not to be seen purely as a forcing action on the larger scale flow; the convection is itself responding to conditions of the large scale flow.

Hurricanes that occur in the Caribbean can sometimes be seen to develop from the easterly waves with their origins over West Africa. In terms of maintenance of the atmospheric global circulation hurricanes are thought not to have a major role. They do, however, have a large local impact and are the most destructive type of weather system. An examination of hurricane records for the past 20 years reveals interesting features. Two thirds of all tropical cyclones form in the northern hemisphere. Tropical cyclones, which may or may not develop full hurricane strength, do not form in the immediate vicinity of the equator, the latitude belts 5°–15° being the preferred regions. In the majority of cases their development is very close to the Inter Tropical Convergence Zone. Their temporal distribution shows preferred periods of development, not just in seasonal terms but on a time scale of 10–20 days, probably in response to variations on the same time scale in the large scale tropical circulation. The specific criteria which influence development of an individual cyclone are seen to be a combination of thermal and dynamical conditions. Thermal instability in the vertical is not the *sine qua non* for cyclone development. Low level cyclonic circulation—and an upper tropospheric counter circulation—are seen to be particularly relevant to development.

The use of numerical models of the atmosphere for weather prediction is now commonplace in many national meteorological services outside the tropics. The application of such models in the tropics is more problematical because of the subtle nature of the balance between wind and pressure fields in equatorial regions. However, using initial conditions derived from GATE data, encouragingly good numerical forecasts out to a few days have been made by groups in both the USA and the UK. It would seem that such forecasts have similar quality, in terms of wave movement and rainfall patterns, as the operational models of middle latitudes. The extent to which parameterisations used in such models are capable of adequately representing sub grid scale processes remains an open question. □

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A hundred years ago

American Jurassic Dinosaurs.—Prof. O. C. Marsh publishes in the current number (November) of the *American Journal of Science and Arts* the principal characters of some new species of dinosaurs. On the flanks of the Rocky Mountains a narrow belt can be traced for several hundred miles, which is always marked by the bones of gigantic dinosaurs. The strata consist mainly of estuary deposits of shale and sandstone, and the horizon is clearly upper Jurassic; the dinosaurian remains in this series of strata are mostly of enormous size, and indicate the largest land animals hitherto known. One new species (*Atlantosaurus immanis*) must have been at least eighty feet in length and several others nearly equalled it in bulk. With these monsters occur the most diminutive dinosaurs yet found, one (Nanosaurus) not being larger than a cat. Some of these new forms differ so widely from typical dinosauria that Prof. Marsh has established a new sub-order to receive them, called Sauropida, from the general character of the feet. They are the least specialised forms of the order, and in some of their characters show such an approach to the mesozoic crocodiles as to suggest a common ancestry at no very remote period. In them the front and hind limbs are nearly equal in size; the feet are plantigrade with five toes on each foot. The carpal and tarsal bones are distinct; the precaudal vertebræ contain large, apparently pneumatic cavities; the sacral vertebræ do not exceed four, and each supports its own transverse process. The public bones unite in front by a ventral symphisis; the limb bones are solid. One of the species described and partly figured in Prof. Marsh's paper is called *Morosaurus grandis*; when alive it was about forty feet in length; it walked on all four legs, was probably very sluggish in its movements, and had a brain proportionally smaller than any known vertebrate.

Prof. Wurtz was charged some time since by the French Minister of Public Instruction, to make an inquiry into the organisation of the laboratories and practical instruction given in the several universities of Germany and Austro-Hungary. Prof. Wurtz accordingly made several journeys to the great seats of learning in these two countries, and the *Journal Officiel* of last Saturday publishes at full length his report. Prof. Wurtz insists strongly on the danger of creating large establishments, where students are taught something of everything, and on the necessity of creating special foci for every large section of experimental science. He shows the advantage of special institutes, and insists upon the organisation of chemical, physical, physiological, anatomical, and pathological institutions such as flourish on the other side of the Rhine.

From *Nature* **19**, 28 November, 76, 87; 1878.

review article

A diagram of evolution

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Recent developments in molecular genetics have made it possible to re-examine the question of the relationship between chromosomes and organisms, and heredity and evolution, in the light of what is now known about the molecular basis of all of them.

OUR methods of studying chromosomes effectively and of breeding organisms experimentally were discovered by separate paths and only later did the two kinds of enquiry combine to reveal the bases of heredity and evolution. For half a century they had proceeded in parallel resting on different assumptions and implying a different understanding of the subject which was now to be called genetics. Thus in 1932 when I came to summarise what was known of the chromosomes I was able to argue a convincing unity in the subject. But beneath this unity I was forced to acknowledge a conflict which the chromosome theory was thought to have excluded. For the chromosomes were seen to have evolved according to rules of their own not clearly related to any properties of the organisms whose heredity they were supposed to be carrying[1].

The development of genetics in the past 40 years has confirmed the unity of the subject but it has scarcely noticed this conflict within it. Over the same period, however, there has been added to the old genetics a new genetics. The microbial materials and molecular equipment allow us to ask again, more searchingly, how chromosomes and organisms, heredity and evolution, are connected and how they are separated in life; what, in short, they mean for one another.

Before the nucleus

Viruses and bacteria are not the precise ancestors of our nuclear and cellular organisms but they show us how such ancestors must have worked in several important respects. In the first place, they show us what must be the chemical basis of both heredity and development in all living things. It lies in the interaction of the two contrasted forms of nucleic acid, DNA and RNA. Evidently RNA arises from DNA in development, thereby creating the organism. But it can exist independently of DNA in the simplest viruses. Therefore in evolution it seems possible that DNA arose from RNA[2].

Second, bacteria have on a smaller scale the same linear arrangement of the nucleotides of DNA as do nuclear organisms. They have pre-nuclear chromosomes. They therefore mutate by the same rearrangements of linear sequences: substitution, duplication or loss, inversion or interchange. They recombine also by breakage and reunion of pieces of chromosomes. But they have a circular form which does not allow either the diploid state, or meiosis or crossing over as known in nuclear organisms. On the contrary their rearrangements arise from processes of conjugation, insertion and infection which are elaborately controlled by the chromosomes, by subordinate bodies, plasmids, episomes and phages, and beyond them by enzymes generated by precise nucleotide sequences or genes[3].

The results of this recombination in bacteria are less regular than in nuclear organisms and probably even more wasteful. They are achieved with a loss of inviable types, some redundancy and editing of nucleotide sequences, and overlap in activities such as are believed to occur at the higher stages of evolution[4].

Our knowledge is defective in these respects but it already reveals a third principle of supreme importance, namely that the chromosomes of bacteria are organised for two adaptive functions: they regulate the metabolism of the bacterium, that is of the organism; they also regulate their own future modes of change, that is the evolution of their heredity. This is the same duality that we already know in the post-nuclear world.

To come to a fourth connection: we cannot be sure how far the transposable elements of nuclear organisms[5] are vestiges of the episomes of bacteria; but we cannot doubt that the circular DNA of bacterial chromosomes corresponds to that of the organellae, the mitochondria[6,7] and plastids,[8] found in later evolution. In the origin of nuclear organisms, it thus seems that the ancestors of the nucleus and of the organellae have entered into symbiosis by infection. As I have argued, infection has become heredity[9]. If this is so, it follows that two stages of chromosome evolution now co-exist in the cells of all nuclear organisms. They also collaborate by rules that are not yet understood. It also follows that this origin has been telescoped with all the other successive and decisive steps in nuclear history, namely the invention of mitosis, meiosis and the alternation of haploid and diploid sexual generations.

Finally, the sequences of nucleotides forming units of activity in the bacterial chromosomes are the first in a hierarchy of other units of activity. For they determine the structure of transfer and messenger RNAs which in turn determine the arrangements of

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amino acids in the structures of proteins[10]. Some of these proteins are immensely durable in evolution. The foundations of chlorophyll, cytochrome C and haemoglobin[11], had evidently been laid in the pre-nuclear stage and their genealogy can be traced through the great groups of plants and animals whose development they underwrote Bacteria already had the machine tools with which the advanced industry of the cell was to be furnished[12].

Nucleus and chromosomes

The chromosome we were for long asked to take as our model was a cylindrical rod seen in fixed and stained cells at one stage of mitosis. Its motionless uniformity utterly contradicted the present and past functions that it was claimed to possess. Observation and experiment have gradually removed this contradiction. The bodies so neatly packaged at metaphase, conceal structures and activities and levels of organisation which we can now usefully survey.

The first example of structure and activity being connected was the 'spindle attachment' or centromere, a body responsible for the movements of each chromosome in cell division. Its functions were too big, too vital or too lethal, to be recognised in a breeding experiment. Yet it turns out to be a gene, one with a repetitive nucleotide structure such that, when broken by misdivision, its functions remain in its parts. The same is true of the nucleolar organiser which assembles RNA and proteins in the resting nucleus[13].

These two structures and activities are immediately necessary for the survival, the one of the chromosome, the other of the nucleus. But both appear in the metaphase chromosome modestly disguised as constrictions. More completely disguised is the differentiation seen in the prophase of meiosis, in the lampbrush chromosomes and in the polytene gland cells of flies. In those cells, the chromomeres, beads on a string, corresponding with the linear arrangement of genes, can be shown to have specific activities in protein production[14]. These activities vary between individual organisms and in the course of development. But they leave no trace in the metaphase uniformity of the living or conventionally fixed chromosome. To get inside the metaphase chromosome we have to use special methods and we then find a special kind of differentiation, not in beads but in blocks or segments.

The first step in tracing a differentiation of chromosomes in blocks was made by Heitz when he claimed that the property of failure to uncoil in the resting nucleus was specific and unconditional in certain segments of chromosome in plants and animals generally. To these segments which he argued were inert he gave the name of heterochromatin in contrast to the bulk of active euchromatin[15]. The second step was to make this resting stage differentiation visible at metaphase. In species of the wood-lily *Trillium* a period of freezing led to the appearance, at metaphase and anaphase of mitosis, of constricted segments. These segments were said to be starved of nucleic acid although the starvation, it now appears, was not of DNA but of the protein and RNA attached to it Similarly at mitosis and meiosis in newts of the genus *Triton*. Soon many genera of plants revealed their starved segments and in doing so showed variation on a scale not previously suspected. Often there was so much variation in the species and consequent heterozygosity in the individual that every plant examined could be distinguished by its own chromosome pattern or map[16].

The effect of starvation was to impair or modify the activity of H-segments (as they became known) in their three main mitotic aspects, reproduction in the resting stage, spiralisation in prophase and division or splitting in anaphase[17,18]. These associated effects were attributed to *allocycly*: the synthesis of heterochromatin DNA at a different phase of the mitotic cycle from that of euchromatin DNA[19]. This supposition was later confirmed by autoradiography[20]. Later an unspiralised H-segment was revealed in the X chromosome of a mammal, the male hamster *Cricetus aureus*, by true starvation[21]. Also H-segments in the bean *Vicia* were shown to be specifically broken by a particular reagent, maleic hydrazide, which presumably interferes with their reproduction[22,23].

A further step in exposing the block-differentiation of chromosomes was made when Caspersson in 1968, again using *Trillium*, found that an acridine derivative, quinacrine, would mark H-segments by differential fluorescence, either enhanced with AT bias or reduced with GC bias[24]. Temperature treatment could now be bypassed and the way was open to use digestion and denaturation before staining as a means of distinguishing the proteins organised by particular H-segments in the metaphase chromosomes. At last in warm-blooded mammals and man, as we had seen long before in plants, individual chromosomes could be recognised and their genetic history traced[25].

These various methods revealed evolutionary contrasts. The first was between the whole complements of species. Within the best known plant genera *Allium*, *Trillium*, and *Fritillaria*, for example, heterochromatin may vary in amount from a trace to a third of the whole complement. Such a wide range suggests a directional trend in evolution in which the chromosome has cut loose from the needs of the organism or its environment[26,27]. The second is between individual chromosomes. For some chromosomes (even X and Y in primates) prove to be unstable within a species or a race while others are stable over whole orders and classes[28].

These discoveries answer some evolutionary questions. But they raise new ones. How far does block or banded differentiation correspond with gene or chromomere structure? Clearly they represent the different needs of chromosome organisation at different stages in the cycle of the cell and of the organism[29–31]. How different these needs are is shown by the conditional behaviour of some heterochromatin. It may vary in relation to stage of development, position in relation to the centromere, haploidy or diploidy or even the action of an adjoining gene[32]. The most striking example of the last two appears in the X chromosomes of man and the mammals; notably in the mosaic coat pattern of the female tortoiseshell cat.

It seems that there is a hierarchy of control mediated in cellular organisms as in bacteria by enzymic messengers operating at many levels between the nucleotide and the whole cell and often canalised along chromosomes[33]. This hierarchy will have to be separately deciphered in many groups of plants and animals. But it already tells us that there is a division of function and hence an opposition of interest in the chromosome between its own needs and those of the cell and the organism. Most significant in the long term are the effects of this division on behaviour at meiosis.

The evolution of the sexual cycle

A sexual cycle of alternate conjugation and meiosis is characteristic of every group of nuclear organisms. Ninety years ago (just when the word chromosome was coming into use) Weismann suggested that this alternation was itself responsible for evolution: it secured a recombination of hereditary differences which would expose the variation of organisms to natural selection[34]. Two kinds of breeding experiment have in the intervening years turned Weismann's speculation into the main instrument of evolutionary thinking.

On the one hand experiments with organisms have shown that conjugation is the means of bringing differences together. And on the other hand experiments with chromosomes have shown that meiosis is the means of pulling them apart. Further both of the two complementary processes have proved to be under genetic control. But beneath this control a third variable is crucial: the time sequence of fertilisation and meiosis which we call the sexual cycle. These three variables are inevitably connected in evolution.

The sexual cycle evidently began in evolution with meiosis coming straight after conjugation as though no diploid mitosis were possible. This haploid supremacy is seen only in the

Protista and lower plants. From it animals seem to have moved abruptly into the opposite state of a complete diploidy; so abruptly that if they stood alone this revolution might have left us nonplussed. But a gamut of transitional stages is preserved in plants. In them meiosis is deferred by a series of steps, which have proved effectively irreversible. In this way the diploid phase has displaced the haploid, finally in a few Protista or Algae even extinguishing it (Fig. 1)[1].

In multicellular organisms, it has long been supposed that the diploid phase has expanded owing to the evolutionary advantage of its enlargement of the range of variation. The difference in its expansion between animals and plants we can now see is due to the different requirements of our second variable, the control of fertilisation, or the restraint of both outbreeding and inbreeding. In animals this control lies almost entirely in the mobile behaviour of diploid organisms. In plants it lies in the protein reactions governing incompatibility in their haploid and diploid phases. These reactions are now thought to have a genetic continuity all the way from their bacterial origins[35]. In the Angiosperms the haploid phase is preserved because in addition it is manifestly indispensible for the mechanisms of the style and of double fertilisation which provide the most exact and versatile of all genetic controls of the breeding system and the one which was evidently responsible for the origin of the angiosperms[36].

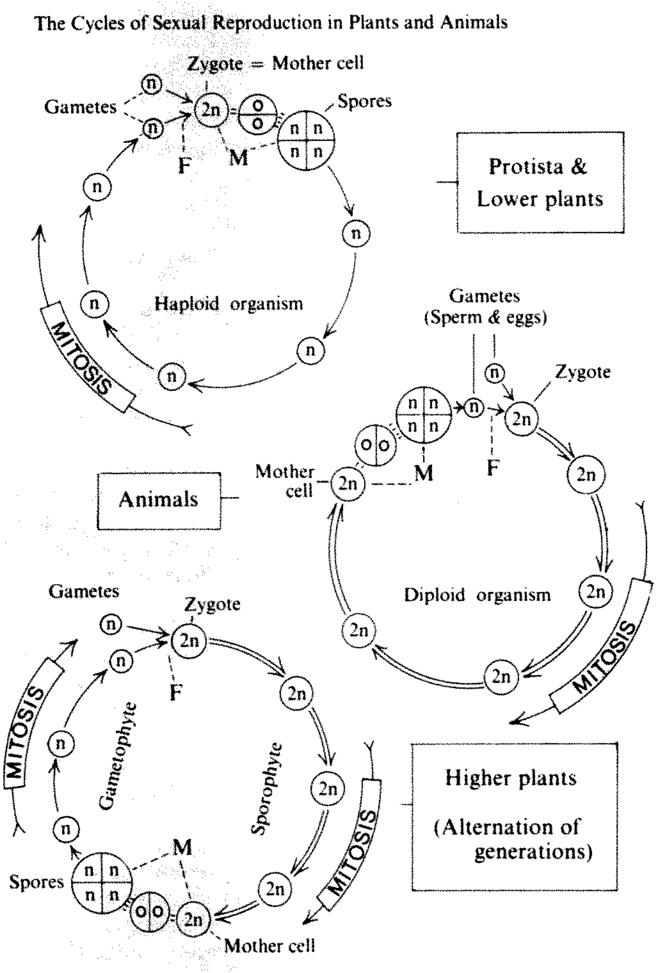

Fig. 1 Diagram showing the three types of sexual cycle found in nuclear organisms to illustrate their evolutionary connections. F, fertilisation or conjugation; M, meiosis; n and 2n, single and double lines, haploid and diploid.

Such is the macro-evolutionary panorama. Behind it however the breeding of organisms and of chromosomes now reveals an array of smaller events, the surviving successes of repeated evolutionary experiments. All of them concern genetic recombination as we can tell when either of the components of sexual reproduction, conjugation or meiosis, fails.

Characteristically in parthenogenetic plants and animals the two processes are suppressed together[37]. But without suppressing either, in hermaphrodite plants a mutation to self-fertility can quickly bring recombination to a standstill. In either case variation ceases. The stock is fixed as a stable race or species, adapted perhaps but no longer adaptable. So long as sexual reproduction continues without inbreeding, however, the option is left open and adaptability is preserved for the future.

Naturally, except in domestication[38], plants and animals as a whole have kept the option open. They are adapted to preserve the future interests of the species. These embrace the need to maintain, in changing environments and in successive generations of organisms, the cooperation of chromosomes and nuclei, mitochondria and plastids, genetic materials and agents, whose relations, as we saw, are relics of the past. When we recall that failure at any point may lead to sterility, a full stop, we understand why this problem of internal adaptation, of mutual selection, baffled Darwin and was avoided by the Darwinians.

The adaptation of meiosis

To understand the taking apart of the differences brought together in the sexual cycle we must take note of the inherent paradoxes of meiosis. For meiosis connects individuals and generations; it also separates them. At meiosis the DNA code is organising both an unlimited continuity of organisms and a repeated discontinuity between them. And in meiosis the chromosomes make themselves the agents of a drastic choice between stability and variation. How do they do it? By a variety of devices modifying meiosis which arise, to use Wilson's phrase, as natural experiments[39]. For they appear in all animal and plant groups throughout evolution without regard to affinities or lineages or levels of organisation.

The most widespread of these devices springs from the earliest manifestation of sexual differentiation itself. In all kinds of organisms whether hermaphrodite or separate-sexed, in which small sperm fertilise large eggs, these germ cells are produced by way of two sizes of cell and hence by way of two forms of meiosis. In its common origin before sexual differentiation this meiosis must have uniformly required pairing, crossing over and chiasmata between parental chromosomes[1]. But one of the two forms, male or female, later often found another way of doing it. Reduction, segregation and recombination were achieved without crossing over, or the formation of chiasmata, or the separation of genes within the chromosome. Thus in one sex, always the XY sex where there was one, never of course in both sexes, whole chromosomes were inherited as a unit[40].

In this way two tracks had been laid in heredity. On one track new chromosomes are constructed by crossing over in every generation. On the other, old chromosomes are kept intact potentially forever. This elegant device was the key to the controlled breeding of *Drosophila* in experiments. But for some time before it had been the key to the breeding of flies in nature and their evolution[41].

The two-track system works in a variety of ways. Meiosis without chiasmata is the simplest. It is found not only in flies but also in mantids and scorpions, moths and mites as well as in certain hermaphrodite plants[42,43]. Haploidy in the male sex is another way of reaching the same goal. It allows bees, wasps and ants, and single species of beetles and bugs, to avoid all recombination in the male sex[13]. But it has one drawback, that these males have no offspring of their own sex. Most widespread of two-track systems is probably that in hermaphrodite plants and in some Amphibia where the large chromosomes allow us to see the frequency and distribution of chiasmata sharply or slightly contrasted between the two sexes[41,44].

The mechanisms of two-track heredity are thus slight or drastic, direct or devious. In them heterochromatin and accessory chromosomes sometimes play a part whose working remains to be explored. But in its consequences (which are its evolutionary causes) two-track heredity is sharply definable. So long as outbreeding is maintained, the choice of track gives the sexual process an unforeseen, and hitherto unrecognised, scope and power. For not only does it generate diversity through the uncertainty it organises in recombination. It also organises its distribution selectively in succeeding generations.

On this basis we can look again at the evolution of sexual reproduction as a whole. Its origin and all its adaptations now show us that it concerns not the organisms in which they began but the selection and survival of their descendants. Never are they concerned with adaptation to any present environment; always either to future environments or to future internal arrangements in the species, sometimes in long hierarchical successions as in, longest of all, the evolution of the life cycle itself.

What has made this possible is the continuity of the chromosomes from generation to generation. Their continuity (or immortality, to use Weismann's term) overrides the discontinuity or individuality of organisms which has over-awed our naturalists: embryologists and paleontologists, Darwinians and Mendelians alike, have taken it as the axiom of axioms. But it is the continuity of the chromosomes which has allowed genetic systems to be built on natural experiments; these experiments have been tested on individuals, but they have been maintained by communities which like the chromosomes are extended in time and space beyond the individual, beyond the organism.

Direction and feedback in selection

Comparisons of organisms in both their living and fossil anatomy have long convinced us that they often show great trends in the evolution of their bodily structure. These trends were first given the name of *orthogenesis* by paleontologists who saw a convenient Lamarckian interpretation for them[45]. They are, however, easily understood as the product of positive feedback in natural selection where one change favours rather than disfavours another in the same direction. If this is so we have to ask whether such trends occur at the other levels of evolutionary change which we now recognise, and with what effect.

In fact we find trends at all our lower levels of evolutionary change. Examples are: repetitive sequences in DNA[46,47]; extreme proliferations of heterochromatin[48], multiplication of chromosomes by polyploidy in plants[38]; by fragmentation in both plants and animals (giving bimodality of chromosome size in many groups)[38,43]; multiplication of accessory and X chromosomes[49–52]; decay of the Y chromosomes[39,49]; expansion of interchange hybridity giving ring formation at meiosis[38,40]; and extravagant incompatibility allelism in plants[35].

With this variety of examples we can now ask another question, whether any connection exists between trends at different levels. The answer is that no such connection seems to exist. A change at one level may no doubt be responsible for initiating a trend at another level, but no more.

Take the polyploidy that is seen in flowering plants. It sometimes leads to a series of multiples: 4, 8, 16, 32 and so on. The scale and range of this occurrence in thousands of genera, is now so well known as to constitute a vast natural experiment[38]. Now polyploid plants arising in cultivation are enlarged in size and plant breeders welcome this gigantism. But in nature the plants themselves do not. On the contrary they either reject it or correct it. Even, we may say, when they reduce their chromosome size, they actively disconnect it from their bodily structure[13].

What then do the polyploid trends achieve?

Evidently each jump has separated a new stock from an old one. By inter-sterility it has initiated a new species isolated genetically from its parent[53]. The same is true in less degree of all changes of chromosome number in both plants and animals. But it is also true that these changes are commoner where the organism is small and short-lived so that the species can afford to experiment; they are less common where the organism is large and its slow propagation makes the gamble more costly. Notably is this so in the flowering plants and in our own group, the primates[54].

Interchanges between chromosomes demonstrate the same principle. How do they establish themselves? In all outbreeding plants and animals migrating colonies are liable to be isolated and so compelled to inbreed. In this emergency parthenogenesis is one means of preserving the reservoir of variation and hybridity they may need for survival. Polyploidy is another. A third is interchange[38,55]. Two chromosomes AB and CD exchange segments to give BC and DA. The nucleus is then hybrid and the chromosomes, old and new, can no longer simply pair. At meiosis they form a ring of four: AB-BC-CD-DA. Such a ring can give viable gametes only of two types, the old and the new, AB, CD and BC, DA. Either of these uniting with a similar partner will give a complete homozygote with impaired viability. But by uniting with the other type it will keep the full heterozygosity of its parent. The interchange hybrid with its ring of four therefore both grows well and breeds true. Further interchanges can enlarge the ring to six, eight and on to fourteen. Each step in this trend feeds back to make another step in the same direction more desirable. Finally all the variation available in the outbred community will be trapped and held in one inbred and now true-breeding hybrid stock or species.

Thus the effect of interchange is to raise barriers to recombination not through crossing but through crossing over, not at the diploid but at the haploid level, and not between organisms but between chromosomes.

The classical instances of this process are the species of *Oenothera* which have been forced into their evolutionary trend by a conversion from outbreeding to inbreeding, itself promoted by migration across North America after the retreat of the ice. De Vries founded his mutation theory on the breeding behaviour of these species and we can now at last do justice to his much-disputed argument[56]. The building up of his hybrid species clearly depended on connecting the two primary initiatives, of genic self-compatibility and chromosome interchange. Both of these were internal or genetic changes. Both we may call mutations. But it was through the breaking down of these hybrid species, their secondary evolution through hybridisation in Europe, that de Vries came on what he called mutations[13].

Oenothera thus shows us both the building up and the breaking down of species. And in both processes we are left agreeing with de Vries that the selection of organisms by adaptation to the environment is secondary to the internal, lower level, processes of mutation. At the same time we discover that the trend in building up interchanges has no connection with any trend in the bodily evolution of the organisms, the plants[38].

Most notable for their evolutionary trends are the sex chromosomes in their XX–XY systems of determination. Both X and Y have two parts, a pairing segment in which the two exchange by crossing over, and a differential segment without crossing over and hence, for the Y, confined to one sex[40]. This restriction as we might expect makes the Y especially vulnerable and its evolutionary disappearance has been independently inferred in most large animal groups[42,43]. Organisms in which the loss has occurred do not however exploit it. On the contrary we again have to say that they correct it. They accept interchange of X or Y with a pair of autosomes. In doing so they often initiate a trend. They build up a ring or chain of chromosomes at meiosis in the heterozygous sex. This process reaches a climax in a number of plant and animals: 3 chromosomes joined in a marsupial[57] 5 chromosomes in a hop[13] 8 in a mistletoe[58] 9 in a centipede,[59] 14 in a termite,[60] 9 or 10 in each of those remarkable relics, the monotreme mammals[61].

In these examples the heterozygous sex extends its fixed hybridity while allowing the homozygous sex to continue its free recombination. Two advantages have thus been combined and reconciled in one sytem: the true-breeding hybridity of *Oenothera* and the two-track heredity of *Drosophila*.

If we look at instability in general at different levels this conclusion is strengthened. Evolutionary similarities of many kinds can now be compared. Organisms provide us with our conventional and systematic standards. The chromosomes show a variety of evolutionary trends. Proteins such as isozymes[62] or haemoglobins[63], and sequences of DNA show much greater stability. But if we look at each group separately we find that each has its own character. As between man and the apes it is the brain that has changed most, the blood and the DNA least, and the chromosomes lie between[47].

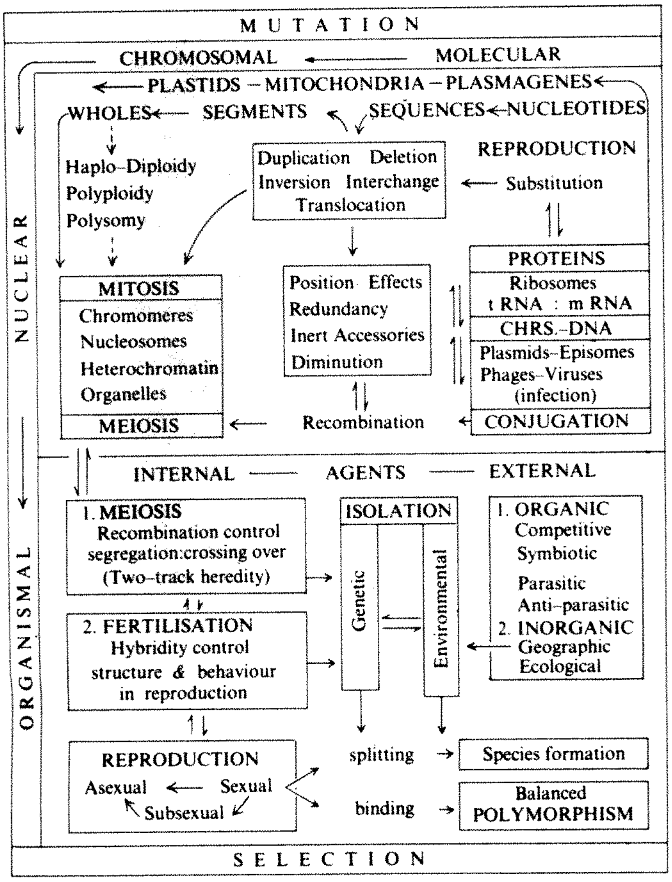

Fig. 2 Diagram showing the multilateral evolutionary relations of molecules, chromosomes, organisms and species subject to mutation at lower levels and selection at all levels.

Although DNA, genes and chromosomes, organisms and species are connected in activity they are separated in selection. It is as though they were protected from one another in stability. We do not know how this happens. But it is utterly clear that if trends at the molecular and chromosome levels are demonstrating feedback in selection so also are trends in the giraffe's neck or the vertebrate's brain. And the Darwinians after all need not have been embarrassed by the notion of orthogenesis[45,64].

The origin of species

The evolution theory began with organisms. For Darwin (in the first and in the last edition of the *Origin*) natural selection worked 'solely by and for the good of each being', that is of each organism[65]. And, if it worked on heredity, that heredity was a property of the organism. In the same vein Morgan wrote that 'the chromosomes fulfill the requirements of genetics' meaning that groups of genes do what organisms require them to do[66,67]. Which is true if we allow that the experimenter is seeing nature back to front.

Again when Darwin wrote that species 'originated' as varieties he meant that they arose from differences between organisms. Haldane therefore, putting Morgan's genes into Darwin's organisms, might claim that differences between varieties were 'generally due to a few genes with relatively large effects' while the chromosomes were sometimes convenient in assembling the genes to make still larger differences between species. In principle the mutation of genes, the spatial isolation of organisms, and the natural selection of one through the other, made varieties into species. If the details might be left in the dark, these were the 'causes of evolution'[64] (see Fig. 2).

At this point, however, a number of details were being brought into the light. Differences between related species sometimes seem to have no adaptive connection with their external environments. Might not sampling errors in mendelian assortment be responsible? In small populations, Sewall Wright suggested, these errors would checker the smooth working of natural selection[68,69]. Slowly we have come to see the scope of this principle. But the sampling we notice concerns not just the organisms and genes of Wright or Haldane but the whole gamut of variation from the nucleotide to the species itself. It is indeed a hierarchy with many interactions which we need to examine.

Every new polyploid begins as the smallest of all populations, a unique individual. And it is cut off from its parental diploid stock, often for ever. It is therefore at once a potential new species. In a different way every new inversion within a chromosome is a unique individual. As a heterozygote it is prevented from crossing over with its sister segment, often for ever. Every new interchange likewise yields a unique individual. All of these establish, by an act of sampling, new populations of chromosomes, and nuclei and organisms. And even when the changed chromosomes lie together with their alternatives in the same cells they are genetically isolated from one another[53,70]. For, in their possibilities of recombination, they might as well be ages or oceans apart.

Here *Oenothera* illustrates both the rule and its exceptions. For, in the hybrid species, the genetic isolation of gametic alternatives is not always for ever. It may break down, by crossing over. It then gives mutations; but, unlike the new mutations of *Drosophila*, they are not always deleterious since they are due to combinations of chromosomes already tried out by selection.

We can now see that all outbreeding species are fields of genetic recombination; that any blocking of recombination splits the species; and its source may be, indeed must be, either external or internal, either environmental or genetic. Further we can sometimes see which it is; but not always, since either may quickly promote the other. We can however see from our experiences of chromosomes that when the initiative is external the populations separated will contain more genetic differences than the naturalist would imagine. But not all such differences will contribute to the splitting of a species. Throughout plants and animals the variation within a species is partly concerned with the opposite task, the long-continuing task of holding the species together.

This principle is true at all our levels of analysis and it is best described in terms of the balanced polymorphisms of Ford. Blood groups, immunity reactions, and mimicry systems in animals and the incompatibility alleles of plants all serve to bind a species together[71]. Our knowledge of heterochromatin bridges the gap between these systems and the sex chromosomes of plants and animals. In the heterozygous sex these in turn may be linked with inversions as well as interchanges. An example is the 'sex-ratio' super-gene in *Drosophila*. The males that carry this floating recessive complex correct the sex-ratio of the population by having an abnormal meiosis which gives largely female progeny. The complex is based on inversions, three of them overlapping, and it is common to a group of many species. It is not as old as the X chromosome but it is clearly older than all the species together that carry it[72].

Consider now the entire range of variation arising in a species at all material levels from the nucleotide to the nucleus. It is evidently available to the species for two kinds of selection and adaptation. These are variously developed and compete with one another. On the one hand, older variations are largely pre-empted as polymorphisms which serve to bind the species together. On the other hand, newer variations are always becoming available which may serve to split it apart. But when this split succeeds it still largely fails to break the continuity of the older polymorphisms.

The competition between these choices means that we have to explore the balance between the present needs of the individual and the future needs of the species, between the internal and the external opportunities of each community. Sometimes as with the origin of the Angiosperms the incompatibility super-gene may have both splitting and binding functions open to it[73]. And sometimes, as in the beginning of man, a mutational initiative in a single cell, by the union of two chromosomes to make one, may decide the origin of a new species or new family[54]. In Fisher's hands natural selection might have seemed to be a truism[74] but these choices and conflicts that are now open to our examination have nothing of the truism about them.

The use of a diagram

Darwinians have seen evolution as the product of mutation and selection. We may agree. But for them mutation, the primary process, acted at the lowest level in their scheme, which came to be known as the 'gene'. And selection, the secondary process, acted, at the highest level in this scheme, that of the organism. For us the physical continuity exists, and the mutation and selection therefore act, at many levels. For us mutation runs all the way from the single nucleotide to the nucleus and the plasmagene. And selection runs all the way from the tri-nucleotide to the community of organisms and, what is more, through a succession of generations. Selection is therefore enormously extended in time and in space.

To use this knowledge we need to take to pieces and reassemble both the assumptions and the conclusions that our predecessors, properly in their time, attempted to establish. We need to think of the relations between levels and systems and in order to do so we need a new framework, a new diagram. Even in its two dimensions such a diagram may help us to see both the connections and the gaps in our understanding.

What we see is heredity changing and using its materials and its processes, derived from the past, to meet the needs of the present and the hazards of the future. It does not show us how often, as in human affairs, the long-term advantage is set aside in favour of short-term opportunity. But it allows us to see how very long the past is both for molecules and for chromosomes. And above all it allows us to see that behind the mendelian choice between change and no-change lie a succession of deeper alternatives within heredity itself: between control by the nucleus and by the cytoplasm; between the fixing or recombining of nucleotides, of genes, of segments and of chromosomes; and between the binding or splitting of sexes, of races and of species.

In all these choices mutations are the basis of future adaptations and evolution. They are pre-adaptations not just one stage but a lengthening succession of stages in advance of the transformations that overtake organisms and communities. Each of the great steps in this process, beginning with RNA and DNA, L-amino acids and proteins, has in turn prescribed some future developments and proscribed others. All have curtailed darwinian randomness. Among the greatest steps of all, some were telescoped in the invention of the nucleus and the nuclear chromosome, mitosis and meiosis; others were fully exposed in the evolving sexual cycles.

When, in 1871, Matthew Arnold suggested that Darwin's ideas were 'all in Lucretius' in one respect he understated the case[75]. To atomists, old and new, changes of atoms were bound from the beginning to have that priority over selection of which Darwin was uncertain[76,77]. And it was only in the last stages of evolution, and only to us today, that the consequences of this priority became unmistakably clear. For the little changes which came near the end gave that genetic control of fertilisation, and through gestation of development, to which first the Angiosperms and then the mammals owed their origins[36,37].

In these processes the forward-looking or experimental capacity of selection culminated. For they allowed heredity to control the environment not only of each organism but also of the following generation. What was in neither Lucretius nor Darwin was the extension of this principle which has been demonstrated by the history of man himself, the extension which allowed individuals to create the environments of indefinitely succeeding generations. Whence sprang a process of directed evolution with a feedback so formidable that it is only now brought within reach of our enquiry[54].

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articles

Eternity is unstable

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We show that if the Universe expands forever, it will become increasingly irregular in the far future. In particular, we demonstrate that the Hawking black hole evaporation process causes a vortical instability to develop in spatially homogeneous spacetimes which are approximately $k=0$ Friedmann universes. We associate the increase of irregularity with the increase of gravitational field entropy, and thus provide a novel picture of the universal heat death.

THE aim of this article is to describe, in the light of recent developments in gravitation physics, the likely future state of the Universe if it expands forever. We shall be especially concerned with extending the analysis of Collins and Hawking (CH), who in their classic paper[1] studied the asymptotic stability of the ever-expanding Friedmann universes under the action of generic, homogeneous perturbations. Their aim was to test the plausibility of Misner's idea that our Universe, now virtually isotropic, could have arisen from an arbitrarily non-uniform set of initial conditions[2,3]; its present regularity being guaranteed solely by the laws of physics with no memory of initial conditions. They examined the most general spectrum of homogeneous perturbations to the flat, ($k=0$), and open ($k=-1$), Friedmann models, and concluded that unless the Universe were neighbouring the special state of zero total energy ($k=0$), the property of isotropy is unstable. Only initial data of zero measure in the metaspace of all possible cosmological Cauchy data give rise to universes of zero total energy which are able to isotropize in the future. In general, the open universes with non-zero total energy will become increasingly anisotropic with the passage of time[4,5]. The Collins–Hawking analysis tells us that either the Universe is young—instabilities having had insufficient time to grow remarkably as yet—or that the Universe is in a highly preferred, zero measure, binding-energy state. They opt for the second alternative by invocation of the anthropic cosmological principle[6–8], which we discuss below.

By extension of the CH theory we shall show that there are novel consequences for the future dynamics and thermodynamics introduced by particle evaporation from black holes[9,10]. It transpires that the non-conservation of baryon number of the gravitational interaction [11] creates an inevitable radiation-filled environment in the far future of a universe which approximates the zero energy Friedmann model. This thermodynamic state renders such universes unstable to the development of vorticity and random peculiar velocities. These universes can no longer isotropise in the manner defined by Collins and Hawking; they become increasingly dominated by the centrifugal and kinetic energy of material motions rather than the particle rest energies and tend towards a dynamical state thay may be viewed as radiation-dominated, relativistic vortex. Thus, the Collins–Hawking proof of the instability of the $k=-1$ Friedmann universe coupled with our proof of the instability of the $k=0$ Friedmann universe shows that all ever-expanding universes are unstable: they do not isotropise at large times.

In the following sections, we first give a short history of the studies dealing with the final state, and then we describe the possible and probable outlines for the classical and quantum evolution of the Universe into the far future. The consequences of this discussion for the stability of Friedmann universes are calculated and some further general ideas regarding the asymptotic behaviour of the Universe ventured in the final section.

The past

Eschatological studies of the Universe have periodically attracted the interest of many physicists, philosophers and astronomers[12,13]. These investigations have, in the main, been precipitated by the second law of thermodynamics which arose as a generalisation of Carnot's work[14] in 1824 on the efficiency of heat engines. In 1852 Lord Kelvin extended Carnot's work and propounded[15] the "universal tendency in nature to the dissipation of mechanical energy". Two years later, Helmholz made the first explicit statement of the now familiar notion of the 'heat death of Universe'. He envisioned an ultimately stable state of all-encompassing thermodynamic equilibrium[16]: "the Universe from that time forward would be condemned to a state of eternal rest". Clausius echoed Helmholz' ideas as follows[17,18]

> "the more the Universe approaches the limiting condition in which the entropy is a maximum, the more do the occasions of further changes diminish; and supposing this condition to be at last completely obtained, no further change could evermore take place, and the Universe would be in a state of unchanging death."

These ideas were widely discussed in the latter half of the nineteenth century and indeed had a profound influence on the *Weltenschauung* of that period[19,20]. Jeans[21] and Eddington[22] were responsible for bringing the notion of the 'heat death' into relativistic cosmology. Eddington asserted in 1931 that[23]

> "It used to be thought that in the end all the matter of the Universe would collect into one rather dense ball at uniform temperature; but the doctrine of spherical space, and more especially the recent results as to the expansion of the Universe, have changed that.... It is widely thought that matter slowly changes into radiation. If so, it would seem that the Universe will ultimately become a ball of radiation growing ever larger, the radiation becoming thinner and passing into longer and longer wavelengths. About every 1,500 million years it will double its radius, and its size will go on expanding in this way in geometrical progression for ever."

Eddington's view of the Heat Death has important similarities with the picture of the Heat Death derived in what follows.

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The classical future

Let us first consider the ultimate fate of the large aggregates of matter that populate the present day Universe. As time passes, clusters, galaxies and other stellar systems will become increasingly bound by gravitational forces since they will radiate away their binding energy in the form of gravitational waves. Dead stars with mass exceeding the Landau–Chandrasekhar limit, $M_{\rm LC} \sim \hbar^{3/2} m_{\rm n}^{-2} \sim 1.5\, M_\odot$ will be the first objects to become black holes, but galaxies and the largest bound configurations $\sim 10^{17}\, M_\odot$ will also eventually follow them. The time taken for the radiation of the binding energy to precipitate clusters into black holes may be roughly determined from the time scale on which these structures will change their quadrapole moments. Equating the gravitational energy[24] $\sim M^2 R^{-1}$ with the rotational energy we obtain from the Landau–Lifshitz radiation formula[25,26] a lifetime $t_{\rm g} \sim G^{-3} c^5 R^4 M^{-3}$ seconds against gravitational wave decay.

The time scale for a large $10^{15}\, M_\odot$ cluster to evolve into a black hole state under the assumptions of these naive arguments is $\sim 10^{19}$ yr.

This radiative loss of binding energy is the principal means whereby the largest aggregates of matter will become increasingly bound into trapped, black hole states. Another is by the 'evaporation' from their interiors of objects which gain sufficient kinetic energy from gravitational deflections, encounters and instabilities to attain escape velocity. Such 'evaporation' will clearly accelerate the central core's collapse to a compact state since objects with positive energy will be diffusively ejected from the system leaving its total energy thus more negative. The details of such mechanisms are exceedingly complex because of subtle collective relaxation effects[27], but the time scale will clearly be related to the relaxation time for gravitational encounters[27–30].

Clusters will for example evaporate galaxy-sized objects in $\sim 10^{11}$ yr and stellar-size ones in $\sim 10^{23}$ yr. Of course, many of the assumptions used to generate these numbers have been stretched to the limit (and beyond) in this context; it serves merely to illustrate that evaporative effects will occur to augment the radiative decay of self-gravitating systems.

Material may be cycled through infall and evaporation many times as the Universe moves inexorably towards a state consisting of supermassive black holes, debris in the form of 'dead' matter, elementary particles, and radiation. The fate of these components will ultimately be determined by quantum processes and we now turn to consider them.

The quantum future

The preceding section traced the evolution of an ever-expanding universe until its matter resided in essentially four basic states: (1) black holes; (2) condensed objects such as neutron stars, black dwarfs, and various clumps of matter; (3) dispersed elementary particles; (4) radiation. However, the evolution of the matter will not stop here. In fact, we wish to argue that material in the first two states will eventually be converted via quantum processes into the third and the fourth types, and that those open universes now neighbouring a $k = 0$ Friedmann universe will eventually become radiation dominated.

Condensed objects of type (2) will be converted into either black holes or dispersed elementary particles by quantum tunneling processes. The zero-point motions of the particles comprising a neutron star or black dwarf could cause the surface of the star to fluctuate inside its Schwarzschild radius[31], or else impart escape velocity to a particle on the stellar surface. If the former does not occur, then the latter process will eventually lead to the complete evaporation of the star: its particles will become completely dispersed[32]. As an example of this dispersal process, consider the decay of a heavy nucleus. Its constituent particles can tunnel through the potential barrier as long as the total mass-energy of the escaping particles is balanced by an increase of nuclear binding-energy, and this will be the case until the nucleus has decayed into ^{56}Fe. As no nucleus has a greater binding energy than ^{56}Fe, further decay can proceed only after nuclear baryons decay, and the decay will be caused by the Hawking process discussed below. The decay of a black dwarf is similar. It will first decay into an iron star, then a neutron star, and then either tunnel into a black hole, or evaporate as its constituent baryons decay.

Hawking has shown[10] that a black hole will emit black body radiation at a temperature which is inversely proportional to its mass. A black hole thus cannot be a final state of matter. Instead, most of a black hole's mass will eventually be turned into radiation, and a small fraction into high-velocity elementary particles. (Copious particle production will not occur until the final stages of the black hole evaporation when the black hole temperature reaches 10^8 degrees, see ref. 33). A black hole will not begin to radiate away its mass until the expansion of the Universe cools the ambient radiation below the black hole surface temperature, but in an ever-expanding Universe, this will inevitably happen. To see this inevitability, note that there are two effects which act to augment the mass of a black hole: the accretion of matter and radiation by a black hole, and the coalescence of black holes into larger ones. The complete evaporation of black holes will occur if the ambient radiation temperature, which is proportional to $1/R$, is in the long run being reduced faster than the two above mentioned effects can reduce the temperature of black holes, which is proportional to $1/M$. Carr[34] has shown that for black holes which are initially smaller than the particle horizon, accretion by matter and radiation in an environment where the average matter density is proportional to the cosmological density cannot increase the black hole mass by more than an order of magnitude in the entire future history of the Universe. At late times, the matter density near a black hole should be proportional to the cosmological density. Furthermore, no black hole whose event horizon does not intersect the initial singularity (that is, non-*ab initio* black holes) can be larger than the particle horizon (this fact can be seen by inspection of the appropriate Penrose diagram). Thus if we make the physically reasonable assumption that there are no *ab initio* black holes, we have a cutoff at high mass in the black hole mass distribution at any given time; the Carr argument then tells us that there is an upper bound to the mass of a black hole, and hence a non-zero lower bound to the black hole temperature, at all times. This assumes, of course, that the black holes cannot coalesce to form black holes of arbitrarily large mass. To calculate the increase of black hole mass due to coalescence, note that the rate of increase of mass due to black hole collisions is proportional to $\sigma_{\rm BH} \rho_{\rm BH} \langle v \rangle$, where $\sigma_{\rm BH}$ is the black hole capture cross-section (this factor includes the potential of the gravitational Coulomb effect to capture other black holes). $\rho_{\rm BH}$ is the black hole mass density, and $\langle v \rangle$ is the average velocity of the black holes with respect to the fundamental observers. Novikov and Thorne[35] have given $\sigma_{\rm BH} \propto M^2 \langle v \rangle^{-2}$, and so we have $\mathrm{d}M/\mathrm{d}t = \rho_{\rm BH} M^2 \langle v \rangle^{-1}$. Now $\rho_{\rm BH} \propto 1/R^3 = 1/t^2$. Assuming $\langle v \rangle \propto t^{-m}$ and integrating, we see that M is bounded above even in the limit as $t \to +\infty$ if $m < 1$, which will hold if $\langle v \rangle \propto 1/R$ or $1/R^{1/2}$. This means that the total number of collisions occuring in infinite time will be finite in the Friedmann universes considered. Thus in any ever-expanding Universe, black holes will eventually completely evaporate; in the far future of an open Universe, all matter will be reduced to elementary particles and radiation.

But the evolution of matter will not cease here. A careful consideration of the Hawking process leads to the conclusion that protons and electrons—supposedly stable elementary particles—are in fact unstable and will decay into massless particles. The 'no-hair' theorem[24] tells us that a black hole is characterised by only three parameters: mass, angular momentum, and electric charge. Hence, there is no way to distinguish between a black hole formed by the collapse of matter from a black hole formed by the collapse of anti-matter. The Hawking process causes an equal number of particles and anti-particles to evaporate from a black hole, so if a black hole which completely evaporates was originally formed of matter, the combined process of black hole formation and evaporation leads to a net decrease in the total baryon and lepton number in the universe. That is, the Hawking process can violate the conservation of baryon and lepton number. Since general relativity contains no intrinsic length scale, black holes of any size can in principle exist; this tells us that the gravitational interaction intrinsically violates lepton and baryon number. (One way of thinking of this is $p+e\rightarrow \mathrm{BH}\rightarrow 2\gamma$). That is, the proton and electron comprising a hydrogen atom can quantum-mechanically tunnel inside their combined Schwarzschild radius to form a black hole, which then decays via the Hawking process into two photons). Some decay modes of electrons and protons via this gravitational interaction are

$$\begin{aligned} p &\rightarrow e^{+}+\pi^{0} \\ p &\rightarrow e^{+}+2\gamma \\ p &\rightarrow e^{+}+\nu+\bar{\nu}. \end{aligned} \tag{1}$$

Protons and electrons are known to be stable within the limits of experimental measurement[36], so the time scale for the gravitational annihilation of baryons and leptons must be extremely long—a rough calculation by Zel'dovich[37,38] gives a half life t_0 of 10^{45} yr. This half life is very small when compared with eternity, however, and all baryons will eventually decay into leptons and radiation. The charged leptons themselves do not decay because the gravitational interaction conserves electric charge.

The baryon mass density will be proportional to $e^{-t/t_0}t^{-N}$ at late times, (at least until the reverse reactions of (1) become important. In the low temperature regime considered here, the reverse reactions will be dominated by the zero point fluctuations considered below) where the value of N depends on the effective equation of state ($N=2$ and $3/2$ for matter-dominated and radiation-dominated cosmologies respectively). The ambient radiation density is proportional to t^{-N}, so the radiation density will eventually dominate the baryon density. Neglecting for the moment the zero-point fluctuation, the material of the Universe at late times will essentially consist of an electron–positron plasma in a radiation bath. One might expect that the electron–positron pairs would rapidly annihilate leaving only radiation, but in an expanding universe, this does not necessarily hold. For a sufficiently rapid expansion, the electron–positron pairs may become the dominant form of matter. The mass density of the electron–positron pairs in a Friedmann universe is given by

$$\frac{\mathrm{d}\rho}{\mathrm{d}t}=\frac{-3\rho}{R}\frac{\mathrm{d}R}{\mathrm{d}t}-\sigma_{\mathrm{Thomson}}\langle v\rangle\rho^{2}-[\text{electrical attraction term}]$$

For all ever-expanding universes, the second term becomes much smaller than the other terms at very late times, (contrast the black hole coalescence effect discussed earlier). For $k=-1$ universes, or universes which approach this class of Friedmann universes in the infinite future limit, the first term dominates and the electron–positron mass density controls the expansion. However, for ever-expanding universes neighbouring a $k=0$ Friedmann universe, the electrical attraction term will cause the electron-positron mass density to decrease faster than the ambient radiation mass density, so that eventually the former can be neglected in comparison with the latter.

The physical mechanism for this is as follows. In $k=0$ Friedmann universes, the fundamental observers have zero gravitational binding energy. This means that electron–positron pairs will become electrically bound—and hence eventually annihilate—if $\frac{1}{2}m\langle v^{2}\rangle$ becomes smaller than the electrical potential energy of a pair (which is proportional to e^{2}/R), where $\langle v^{2}\rangle$ is the mean square velocity of an electron or positron with respect to the fundamental observers. Now $\langle v^{2}\rangle\propto 1/R^{2}$, and so the electrical potential energy of a pair will eventually become larger than the kinetic energy—an electron–positron pair will become bound. The time needed to annihilate will depend on the initial distance at which the particles first become bound, and so if the initial spectrum of velocities is either maxwellian or determined by the decay of protons via the Hawking process (the number density of protons being proportional to $e^{-t/t_0}t^{-N}$) the mass density of electron–positron pairs will decrease according to $t^{\beta}e^{-t/t_w}$, for some constants β, t_w. Note that this is not inconsistent with the fact discussed earlier whereby black holes undergo only a finite number of collisions in infinite time. It is only necessary that an electron–positron pair undergo one collision in its history for annihilation.

At very late times, then, the universe near the $k=0$ Friedmann will have material consisting of radiation, reactions such as (1) and their reverse, plus the reactions

$$\begin{aligned} e^{+}+e^{-} &\rightleftharpoons 2\gamma \\ e^{+}+e^{-} &\leftrightharpoons \nu+\bar{\nu} \end{aligned} \tag{2}$$

and the zero-point energy. If we include the zero-point contributions to (1) and (2) in the zero-point energy, we see that reactions (1) and (2) go from left to right—from matter to radiation—because the number of massless particles with energies sufficient to produce an electron-positron pair, say, is proportional to the Boltzmann factor $e^{-1/T}$, and $T\propto t^{-N}$, since the expansion is cooling the ambient radiation. The reaction from left to right, on the other hand, is proportional to the particle number density, which is decreasing according to t^{-N}. Thus at very late time the matter will primarily be radiation and zero-point energy.

The effective stress-energy tensor of the zero-point energy has been recently calculated by Davies *et al.*[4,5]. For example the vacuum energy density for massless fields in a flat background evolves as $\rho_{\mathrm{vac}}\sim t^{-4}$ and decays as $\rho_{\mathrm{vac}}\sim R^{-8}$ or $\sim R^{-6}$ in radiation and matter dominated universes respectively.

The calculation of a realistic zero-point energy equation of state would involve numerous interacting fields, and would be too difficult to carry out. However, it seems reasonable to assume that the zero-point energy of the interacting fields at very late times in an expanding universe would be that of those particles which would be final state particles in the absence of the zero-point energy. We have argued above that these particles would be massless particles of various types (photons, neutrinos, gravitons), and so the effective vacuum energy would be negligible compared with the radiation background at very late times. Thus a universe neighbouring a flat Friedmann universe will be dominated by radiation at late times.

The dynamical future

We shall summarise some of the assumptions and conclusions of Collins and Hawking and discuss certain other points of the general and technical interest in their calculation before seeing how they are significantly modified in the light of Hawking's subsequent[10] and remarkable discovery of the quantum mechanical evaporation of black holes and accompanying baryon non-conservation[11]. We have just argued that these processes will ensure that the asymptotic environment of the Universe will be that of a radiation gas in the marginally bound case.

Collins and Hawking were the first to attempt to frame a precise definition of isotropisation for a homogeneous cosmology; a model was said to isotropise if, as $t\rightarrow\infty$, it satisfies the following

I1: $V=R^{3}\rightarrow\infty$, where V is the co-moving volume.
I2: $T^{00}>0$ and $T^{0i}/T^{00}\sim v^{i}\rightarrow 0$, where $T^{\mu\nu}$ is the stress energy tensor; v, velocity field of the matter.
I3: $\sigma/\theta\rightarrow 0$, where σ is the kinematic shear and $\theta=\dot{V}/V$.
I4: $\beta\rightarrow\beta_0$, where $\beta=\int_0^t\sigma\,\mathrm{d}t$ and $\dot{\beta}_0=0$.

Condition I1 aims to exclude closed universes (those having compact, spatial three-slices of homogeneity). Another criterion must be used in homogeneous models that recollapse; one proposal[39] gauges the isotropisation by measuring the degree of isotropy in the spatial three-curvature at maximum volume. Condition I2 of the Collins–Hawking criteria requires the peculiar velocity of matter motion relative to the surfaces of homogeneity to die away in time, whilst conditions I3 and I4 seek to exclude those models in which the shear distortion decays more slowly than the universe is expanding since this would build up a large cumulative distortion with increasing time. Collins and Hawking also make use of two general constraints on the matter fields within the cosmologies. The first, the dominant energy condition (DEC)[40], requires $T^{00} \geqslant |T^{ab}|$ whilst the second, the positive pressure criterion (PPC)[41] requires $\sum_{k=0}^{3} T_{kk} \geqslant 0$. Although the first of these will be microscopically violated in the quantum processes[42] described above, it should still be valid 'on the average' or on large spatial scales. Collins and Hawking consider the most general homogeneous perturbations to the flat, ($k = 0$), and open ($k = -1$) Friedmann space-times. the most general is of the Bianchi VII$_h$ type requiring one more arbitrary constant to specify the metric than in the type VII$_0$ case which generalises the $k = 0$ isotropic model and is a special case of the VII$_h$ generalisation of an open, isotropic universe. The Collins–Hawking analysis yields:

> Theorem 1 (ref. 1): If DEC and PPC are satisfied then the set of homogeneous initial data giving rise to models which approach isotropy is of measure zero in the space of homogeneous data.

That is, as defined by I1–I4 isotropy is in general an unstable property of an expanding universe independent of its equation of state. In proving this theorem they assumed that conditions I1 and I3 held and showed that this implied I3 and I4 could not. This tells us that the inevitable instability revealed by their theorem is associated with a non-decaying shear mode violating I3 and/or I4. In fact, examination of the VII$_h$ model close to isotropy reveals $\sigma/\theta \rightarrow$ constant as $t \rightarrow +\infty$. Thus, $\beta \sim t$ and an ongoing cumulative distortion arises to first order. There could of course also exist in the VII$_h$ perturbation a vortical instability violating I2 which is explicitly suppressed by the method of proof.

Next, Collins and Hawking fix on a preferred subset of cosmologies—those in the neighbourhood of the zero energy ($k = 0$) models and their homogeneous perturbations of type VII$_0$. In this case the behaviour qualitatively changes since the stability is in fact sensitively dependent upon the particular matter content of the space-time—as might be anticipated since these models, by definition, contain sufficient matter to influence and balance the expansion energy at all times. (In the open VII$_h$ models the asymptotic behaviour is dominated by kinematic and geometrical effects and all memory of realistic matter fields is lost as $t \rightarrow \infty$.) In order to arrive at a strong result regarding zero energy universes CH make the strong assumption that the matter content of the unperturbed isotropic model be just pressureless dust, ($p = 0$ state), and that traceless radiation fluids enter only as components of the perturbed matter currents. With this addition they prove:

> Theorem 2 (ref. 1): Let S be the subspace of type VII$_0$ initial data in the space of all homogeneous initial data. If the unperturbed Friedmann model contains dust matter and the perturbed matter content be comprised of dust and zero rest matter with tracefree stress tensor satisfying DEC, then, there exists an open neighbourhood of the $k = 0$ Friedmann initial data such that all data in this neighbourhood give rise to models which isotropise.

Thus, if a dust-filled universe lies sufficiently close to a state of zero total energy then it is stable against the development of anisotropy via homogeneous gravitational wave modes concordant with its global topology. (There may, of course, exist additional inhomogeneous gravitational wave perturbations to which it is unstable; see Bonnor[43,44], but his models are not general.)

From theorems 1 and 2 Collins and Hawking could draw either of two conclusions. Either: (i) The Universe is young; it is not of zero measure and is presently anisotropising under the action of generic homogeneous perturbations which have had, as yet, insufficient time to grow noticeably out of the linear regime. Or: (ii) the Universe is in the neighbourhood of the zero total energy state, the most general homogeneous perturbations admitted by its geometry are of Bianchi type VII$_0$ and decay in time. The Universe is thus isotropising but is of zero measure in the metaspace of all possible cosmological initial data sets. Collins and Hawking prefer option (ii) and justify its relative unlikelihood by appeal to an anthropic[6–8] argument to the effect that galaxies, and hence life supporting environments rich in biologically advantageous heavy elements, would only be expected to arise from such special intial data sets. Conclusion (i) was regarded as unsavoury because it requires our existence as observers to arise in a special interval of cosmic time before the anisotropy has a chance to grow large. Such an objection seems inconsistent since the same 'anthropic' principle used to explain the special binding energy necessary to support (ii) would also defend this no less special assumption required to uphold conclusion (i). Also the arguments invoked to incorporate the Anthropic Principle seem unconvincing given our present knowledge of galaxy formation. For, we might claim, the main problem in explaining the presence of protogalaxies by quasi-statistical fluctuations from homogeneity in the initial state is that these fluctuations do not appear to grow fast enough to give a self-consistent explanation for galaxy formation in any Friedmann universe, whatever its binding energy. To provide understanding of the later nonlinear stages of galaxy formation this difficulty is usually side-stepped and appropriate initial amplitudes simply postulated at some *ad hoc* initial epoch which will guarantee the present structures. Such a procedure really explains nothing of course. *Ad hoc* initial amplitudes will indeed allow flat Friedmann universes to produce bound inhomogeneities by the present epoch as Collins and Hawking wish but although the growth of relative overdensities is exceedingly faster (slower) in closed (open) background universes, the presence of galaxies in these backgrounds can also be explained by initially larger (smaller), but equally *ad hoc*, initial fluctuations.

Before examining the stability of Friedmann universes in more detail, we harmonise two apparently contradictory results, which might otherwise lead to confusion. In an earlier paper Collins and Hawking[45] also examined the behaviour of homogeneous, gravitational wave perturbations to Friedmann models and deduced the precise constraints placed upon the present shear and vorticity of the Universe by the microwave background isotropy over large angular scales. In performing this analysis they showed there exists a shear distortion mode growing in time $(\sigma/\theta \sim t^{2/3})$ if the Bianchi VII$_0$ model is linearised about the flat Friedmann universe. This appears to explicitly contradict theorem (2) of Collins and Hawking quoted above in the light of their isotropisation condition I3 ($\sigma/\theta \rightarrow 0$ as $t \rightarrow \infty$). However, a closer examination reveals that neither conclusion need be in error. In this earlier paper Collins and Hawking[45] discover the following unstable Bianchi VII$_0$ shear distortion mode to a flat, dust-filled Friedmann model:-

$$\begin{gathered}\sigma_{32} \sim \int \tau^{-\frac{1}{2}}[DJ_{3/2}(2\tau) + EJ_{-3/2}(2\tau)]\,\mathrm{d}t \\ \theta \sim t^{-1} \text{ where } \tau \sim t^{1/3} \text{ and } \dot{D} = 0 = \dot{E};\ `.\text{'} = \mathrm{d}/\mathrm{d}t\end{gathered} \tag{2}$$

To exhibit the short time behaviour of this mode we may use the standard small parameter expansion of the Bessel functions to obtain to first order;-

$$\sigma/\theta \sim At^{2/3} + Bt^{-1};\ \dot{A} = 0 = \dot{B}$$

and there indeed appears an anisotropising A-mode of VII$_0$ type close to the flat isotropic model over short times. However, the presence of the Bessel functions in (3) indicates that the shear mode is oscillatory over large time intervals. Using the boundedness of the oscillatory component we do indeed find the VII$_0$ model ($p = 0$), to tend asymptotically to the flat Friedmann model over large times and

$$\sigma/\theta \sim \alpha t^{-2/3} + \beta t^{-1};\ \dot{\alpha} = 0 = \dot{\beta} \quad \text{for } p = 0.$$

The short time expansion used in ref. 45 thus traces the initial growing phase of an oscillatory but asymptotically decaying function.

We now turn to consider the new consequences for the Collins–Hawking analyses from our arguments above regarding the inevitable asymptotic radiation domination of a marginally bound universe in the far future because of baryon non-conservation by the gravitational interaction. This significantly alters the conclusions drawn from theorem (2) of Collins and Hawking regarding the asymptotic dynamical state of universes close to zero total energy undergoing generic homogeneous perturbations. These universes will no longer isotropise, but in general will become vortically unstable at late times. To understand intuitively why this is so consider the newtonian dynamics of a ball of expanding fluid with radius R subject to a small rotational perturbation of angular velocity ω. The angular momentum of this expanding ball is given by $J = I\omega \sim MR^2\omega \sim (\rho R^3)R^2\omega$. Now for a perfect fluid with equation of state $p = (\gamma - 1)\rho$, conservation of mass requires $\rho \sim R^{-3\gamma}$ and thus $\dot{J} = 0$ requires $\omega \sim R^{3\gamma-5}$ with the associated linear velocity evolving as the ball expands as $v \sim R\omega \sim R^{3\gamma-4}$. Thus for newtonian ever-expanding universes filled with radiation $(\gamma = 4/3)$ the velocity, $\dot{v}$, approaches a constant value, violating isotropisation condition 12 of Collins and Hawking. However, this is in fact, physically, a growing instability. Inspection of the Raychaudhuri equation[46,47] governing the general kinematics of the fluid ball is (in the relativistic or newtonian case):

$$3\frac{\ddot{R}}{R} = 2(\omega^2 - \sigma^2) + \nabla_a \cdot A_a - \frac{1}{2}(\rho + 3p)$$

(where A_a is the fluid acceleration), showing that the dynamical influence of the vorticity is gauged by the contrast parameter $\omega^2\rho^{-1}$ which expresses the ratio of the centrifugal energy driving distortions to the isotropy-inducing matter stresses, We have, for small anisotropies, $\omega^2\rho^{-1} \sim R^{9\gamma-10}$. So, for a radiation fluid, (just $p \geqslant \rho/9$ on average suffices), a growing vortical instability develops as the fluid expands which will create a pancake-like or cigar-like configuration and rapidly increasing deviation from sphericity when $\omega R/\dot{R} \sim 1$. A newtonian universe with near radiation matter content would become increasingly dominated by the energy of random and rotational motions rather than the inertia of its rest-mass content. This is in complete contrast to the fate of a dust, $(\gamma = 1)$, ball where the vortical effects die away as $\omega^2\rho^{-1} \sim R^{-1}$ with the expansion. We shall confirm this behaviour with a fully relativistic derivation below, in which case the ball of fluid will be infinite. This simple argument also exhibits certain weaknesses of the CH isotropisation conditions. For instance, condition I2 allows a model to be classed as isotropising if $v \to 0$ as $t \to \infty$ even though $\int v\,dt \to \infty$ and thus the cumulative distortion from the velocity or vorticity perturbations leads to an aspherical configuration. We really require a stronger criterion: that the cumulative effect of the vorticity is non-increasing. By analogy with I4, a possible criterion would be

$$\Omega = \int_0^t \omega\, dt \to \lambda \text{ as } t \to \infty;\ \dot{\lambda} = 0.$$

For example, in a universe close to the flat, Friedmann model $R \sim t^{2/3\gamma}$, and conservation of angular momentum indicates that $\Omega \sim t^{(9\gamma-10)/3\gamma}$. Vortical effects will not lead to cumulative distortions if $\gamma < 10/9$ in agreement with the other criterion used above to discuss vortical instability, namely that $\omega^2/\rho \sim (\omega/\theta)^2$ decay as $t \to \infty$ for isotropisation, (this condition is analogous to condition I3 on the shear). These results indicate that there exist homogeneously expanding newtonian universes which may become increasingly far from isotropy as $t \to \infty$ due to their vortical instability and yet be classified as 'isotropising' by the Collins–Hawking criteria. Furthermore, flat newtonian universes may exhibit vortical instability whenever their fluid content satisfies $p > \rho/9$ and in particular radiation dominated universes are unstable.

It can be demonstrated that this vortical instability arises also in relativistic cosmology, as one might anticipate, since it is a consequence only of a fundamental invariance principle—the conservation of angular momentum. For concreteness we consider the homogeneous Bianchi VII$_0$ model (which generalises the flat Friedmann model) filled with radiation to demonstrate the instability; but the instability exists for all $p \geqslant \rho/9$ fluids. By vorticity we shall mean the rotation of matter about an observer moving with the matter, relative to an inertial frame defined by gyroscopes. the peculiar velocity will represent the velocity of the matter relative to the surfaces of constant homogeneity. We use the notation of Collins and Hawking[1] with our $R = e^{\alpha}$ and $\rho = \mu$ as scale factor and density.

A homogeneous cosmological model is defined as one which admits a three-parameter group of isometries, simply transitive on a family of space-like hypersurfaces[48]. Collins and Hawking[1] label these surfaces by t chosen so that $g^{\mu\nu}t;_\mu t;_\nu = -1$ and the metric has the form $g_{\mu\nu} = -n_\mu n_\nu + g_{AB}E^A_\mu E^B_\nu$ where $g_{AB} = R^2(e^{2\beta})_{AB}$ with β trace-free and $n_\nu = -t;_\nu$ is normal to the surfaces of homogeneity (Greek indices run 0–3 and Latin 1–3). Thus, spatial homogeneity implies $\rho;_\nu = -\rho n_\nu$ for a scalar, ρ. Consider a radiation fluid with

$$T^{\mu\nu} = \tfrac{4}{3}\rho U^\mu U^\nu + \tfrac{1}{3}\rho g^{\mu\nu}$$

The conservation equation $T^{0\nu};_\nu = 0$ yields

$$[\rho(U^0)^{4/3}R^4]^{\cdot} = -\tfrac{4}{3}\rho(U^0)^{1/3}C^A_{AB}U_C R^2(e^{-2\beta})_{BC} \qquad (4)$$

where C^A_{CB} are the structure constants of the homogeneous, space-group symmetries. The acceleration by the fluid pressure is given by another conservation equation as

$$\tfrac{4}{3}\rho U^\mu{}_{;\nu}U^\nu = -\tfrac{1}{3}\rho_{;\nu}h^{\mu\nu} \quad \text{where } h^{\mu\nu} = g^{\mu\nu} + U^\mu U^\nu$$

so:

$$[\rho^{1/4}U_A]^{\cdot} = \rho^{1/4}(U^0)^{-1}C^B_{CA}U_B U_D R^{-2}(e^{-2\beta})_{CD} \qquad (5)$$

The components of the vorticity vector are defined as $\omega^\mu = \eta^{\mu\nu\lambda\rho}U_\nu U_{\lambda;\rho}$ where $\eta^{\mu\nu\lambda\rho}$ is the alternating tensor. Thus,

$$\omega^A = R^{-3}\varepsilon_{ABC}[\tfrac{1}{2}C^D_{BC}U_D U^0 + U_B\dot{U}_C] \qquad (6)$$

and

$$\omega^0 = \tfrac{1}{2}R^{-3}\varepsilon_{ABC}[C^D_{BC}U_A U_D]. \qquad (7)$$

The Bianchi VII$_0$ model generalising the flat Friedmann universe, is of Behr-Ellis-MacCallum[49,50] class A having $C^A_{AC} = 0$. Thus, from (5) for small β, close to isotropy we have:-

$$(\rho^{1/4}U_A)^{\cdot} \sim \rho^{1/4}C^B_{CA}U_B U_C R^{-2}$$

(4) implies $\rho \sim R^{-4}$ thus,

$$(R^{-1}U_A)^{\cdot} \sim R^{-3}C^B_{CA}U_B U_C \sim R^{-3}[1 + 0(v^2)]$$

Thus to first order, perturbations in velocity and vorticity of this type evolve from the Friedmann background as $U_A \sim R$ giving the three-velocity squared as $v^2 \sim U_A U^A \sim$ constant and to first order for the vorticity vector, from (6) and (7) we have,

$$\omega^A \sim R^{-3}[\text{const.}\, R + RR] \sim R^{-2}$$

$$\omega^0 \sim R^{-3}[\text{const.}\, R^2] \sim R^{-1}$$

Thus, the vorticity scalar evolves $\omega^2 \sim R^{-2}$ in agreement with the simple newtonian arguments given above. Modifications due to nonlinear coupling with the shear are to be expected when the vorticity becomes large. Therefore, the flat, radiation-filled Friedmann universe will be unstable to the development of vorticity; this behaviour is not an artefact of the homogeneity of the perturbations and may be recovered from studies of inhomogeneous vector perturbations to isotropy[51]. Intuitively, we may see this from Raychaudhuri's equation which shows that when baryons and leptons have been eliminated by the mechanisms described in the last section the terms driving the expansion are $\rho_\gamma \sim R^{-4}$, the shear $\sigma^2 \sim R^{-6}$ and the rotational energy $\omega^2 \sim R^{-2}$ in the linear regime. For large R the rotational energy clearly dominates. In models with open spatial sections $(k = -1)$, (type VII$_h$ being the most general homogeneous example containing the Friedmann, open model), such an effect also competes with the spatial curvature terms $\sim R^{-2}$ and the relative strengths of the curvature and rotation terms will determine the asymptotic behaviour. If the curvature effects dominate as $R \rightarrow \infty$ they will suppress the vortical instability and allow the shear instability $\sigma/\theta \rightarrow$ constant found by Collins and Hawking to control the future evolution. However, it is still possible for the spin to significantly determine the final state via its non-linear interaction, see for example refs 52, 53. This completes the argument that the 'heat death of the Universe' is sufficient to ensure all isotropic universes are unstable irrespective of their binding energy. The fate of perturbations to closed Friedmann universes was not considered by Collins and Hawking in ref. 1 but other studies indicate that the general homogeneous closed universe of Bianchi type IX does not 'isotropise' in any meaningful sense[39] as it approaches maximum volume. For instance, the spatial curvature does not tend to directionally equalise and will produce anisotropy in the Hubble rates.

Another conceivable cosmological artefact of eschatological significance is the possibility of a non-zero cosmological constant Λ whose presence introduces terms of the order of ΛR^{-2} in the energy evolution equations. This is of the same order as the vortical energy term ω^2 asymptotically in the radiation-dominated universe. We might thus suspect that in the $\Lambda > 0$ case, allowing ever-expanding universes, the isotropic stress induced by the presence of this hypothetical cosmic force field might be of sufficient magnitude to dominate the vortical energy. A negative Λ would essentially reproduce the closed universe evolution since it has been shown[54] that a space-time with $\Lambda < 0$ cannot expand forever: all timelike curves have a finite length $< 2\pi(3/|\Lambda|)^{1/2}$.

It is possible that the collective gravitational effects of density perturbations could be the predominant agent in driving the cosmological flow from homogeneity and isotropy in the large at late times. For example, an irregularity spectrum $\delta\rho/\rho \sim M^{-\alpha}$ contributes to the metric distortions as $\delta g/g \sim M^{(2/3-\alpha)}$. The preferred $\alpha = 2/3$ Zeldovich spectrum[32,55] is envisaged with metric perturbations constant on all scales as they enter the horizon. If a 'white noise', $\alpha = 1/2$, spectrum prevails then they would eventually dominate the dynamics as $M \rightarrow \infty$ and couple with the vortical motions to determine the asymptotic behaviour. It is not know, of course, if such spectra really do extrapolate to indefinitely large mass scales.

We have in this section completed a proof that physically realistic ever-expanding universes asymptotically deviate from isotropy—eternity is unstable.

Asymptotia

The future state of non-flat, ever-expanding universes is characterised[1] by a cumulative shear distortion in the expansion flow. That of the ever-expanding flat models is analagous to a relativistic vortex, for the dominant instability is due to the vorticity of the matter flow lines. In both cases the final state is characterised by increasing irregularity. However, these irregularities will not in general result in a breakdown of the global causal structure in the large; a generic homogeneous space-time is globally hyperbolic, which means that the future is determined by the initial data on some space-like hypersurface. Siklos has shown[56] that homogeneous space-times which violate global hypergolicity have two fewer free parameters than the most general spatially homogeneous space-times. He also showed that type VII_0 space-times which satisfy $R_{ab}K^aK^b > 0$ for all null vectors K^a cannot violate global hyperbolicity, and this inequality would hold in a VII_0 space-time which is dominated by randomly orientated radiation.

Although global hyperbolicity will hold on a large scale, it will be violated in the small in an ever-expanding universe. The complete evaporation of a black hole will generate a naked singularity[57] and this necessarily means a violation of global hyperbolicity. Thus, if one considers global hyperbolicity—the ability to predict the future—to be a property which must hold, even in the small, in any physically realistic space-time, then one is forced to conclude that the actual Universe is closed. Only in this case can an all-encompassing, final singularity intervene to prevent the inevitable formation of naked singularities from black hole evaporation.

The matter vorticity couples to the Weyl tensor in the second order[50], so the instabilities we have discovered and those of Collins and Hawking[1] imply that the Weyl tensor grows with time. Now Penrose has suggested[58,59] that the entropy of the gravitational field is proportional to "some suitable integrated measure of the size of the Weyl curvature". If this connection between the gravitational entropy and the Weyl tensor holds, then our results can be interpreted as saying that the entropy of the gravitational field increases with time. This is just what we would expect; the entropy of all fields should increase with time, unless the decrease of the entropy of one field is more than counter-balanced by the increase in the entropy of another. An asymptotic approach to a Friedmann space-time, which is the future behaviour of the universe envisioned by many cosmologists—in particular by Collins and Hawking[1]—would constitute a decrease in the gravitational entropy without a corresponding increase in the entropy of the other fields. The second law of thermodynamics tells us that this cannot happen (statistically speaking, it must be very improbable) and this is what we have found.

We have arrived at a final state for an ever-expanding universe which is somewhat different from the "state of eternal rest" pictured by the nineteenth century physicists. In our view the space-time geometry is becoming more and more irregular at very late times. Both pictures envision an asymptotic approach to a state of maximum entropy; our version of the heat death is different because we have included the gravitational entropy.

Time is a measure of change, and both versions of the heat death have fewer and fewer changes as asymptotia is approached. Thus, one might expect that physical time could slow down relative to proper time in the far future. Although the asymptotic state is reached only after infinite proper time in an ever-expanding universe, it is possible that it would be reached in *finite* physical time. Misner has argued[60] that proper time is an inappropriate measure of physical time near a singularity. Because changes occur with great rapidity (as measured in proper time), Misner contends that the singularity should have occurred in the infinite past as measured in physical time, though only about twenty thousand million years ago in proper time. A mathematically appealing timescale which has this 'Misner time property' of placing singularities at temporal infinity is the so-called York extrinsic time[24,61] York[61] has proposed foliating a space-time with spacelike hypersurfaces of constant mean extrinsic curvature, and defining time to be given by $T = -\frac{4}{3}K^a_a$, where K_{ab} is the extrinsic curvature of the hypersurface. ($K^a_a = K^1_1 + K^2_2 + K^3_3$ is the mean curvature: the sign is chosen as to make T increase to the future, and the factor 4/3 simplifies the expression of the dynamical Einstein equations). Such a foliation can be shown to exist in a fairly general class of closed universes[62]; and in closed universes, the foliation will also be unique[62,63]. If this foliation by constant mean curvature hypersurfaces exists and is unique in open universes also, then it would define an absolute time for the Universe. In ever-expanding universes with an initial singularity the York time varies between $-\infty$ at the singularity and zero at asymptotia. Hence if York time measures physical time in the far future, then an ever-expanding universe, which exists forever in future proper time, will continue to exist for only a finite physical time. Vaguely speaking, only a finite number of changes will occur in the future; the heat death puts an end to change.

Note that in closed universes, which have both initial and final singularities, York time varies[62] between $-\infty$ and $+\infty$. Closed universes thus have a finite future in proper time, but an infinite future in York time. If York time is indeed the appropriate measure of physical time near the final singularity, then the infinite future duration of a closed universe can be interpreted as saying that the gravitational field provides an unlimited source of free energy near the final singularity, and this permits an infinite number of changes—physical time intervals—to occur.

We close with a final speculation. One of the fundamental problems of statistical mechanics is accounting for the global increase of entropy without introducing a very strong stochastic assumption, such as the postulate of random phases or the assumption of coarse-graining, which is difficult to justify. As discussed in detail by Brush[19], the justification of this type of stochastic assumption has a subjective flavour about it. Coarse-graining, for example, generally requires an appeal to human inability to measure a difference between certain classes of states[64]. What is required is a proof of entropy increase which does not require such a stochastic assumption but uses only the equations of motion of the existing fields and particles (all time-reversible except for the super-weak T-violating interaction) and the universal initial conditions. Penrose's suggested connection between gravitational entropy and the Weyl tensor may provide a mechanism for such a proof. The present article and the work of Collins and Hawking indicate (but do not prove) that in homogeneous ever-expanding universes, the Weyl tensor will increase as one moves far away from a generic, conformally flat singularity. Thus, the increase of Penrose gravitational entropy follows from assumptions about the Initial Conditions: the initial singularity is both generic and conformally flat. Is the Penrose entropy the ultimate source of all entropy increase?

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Punctuated evolution of tectonic style

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A continuous secular evolution in the vigour of mantle convection may have translated into a series of distinct stages in crustal tectonic style.

THERE is much debate about the nature and relative magnitude of the forces responsible for the motion of lithospheric plates[1]. The possibilities considered include the viscous drag of convecting asthenosphere and the gravitational push away from oceanic ridges, but it is the gravitational pull, or negative buoyancy, of the cold, subducting lithospheric slab which emerges as a major[2,3], if not the predominant[4–6], force. The age of crust being subducted today ranges from 5 to 175 Myr (ref. 6) and Atlantic oceanic crust ~200 Myr old has not even started, so clearly achieving a critical value of negative buoyancy is not the only cause of subduction. Some combination of these forces, and perhaps circumstances such as convergence and over-riding of ridge by continent, must be enough to trigger subduction, and cause the spectrum of subducting-crust ages. Nevertheless, if it is accepted that on a world-wide basis, negative buoyancy is the major force in this process, then this view has important ramifications with respect to Precambrian tectonic style.

Buoyancy

From a plate-tectonic point of view, buoyancy is measured relative to the average density of the asthenosphere. Crust, whether it is basaltic or granitic, is positively buoyant, whereas the cold peridotitic part of the lithosphere is denser than the hot asthenosphere. Overall negative buoyancy of lithosphere results from the cooling of oceanic plates as they recede from ridges, and the denser, mantle part of the lithosphere thickens relative to the thin positively buoyant crust.

If higher average heat flow implies a thinner lithosphere, then negative buoyancy must have been less important in the past. Consideration of the possible previous magnitude of the terrestrial heat flux[7], forces associated with earlier, more vigorous convections[8], and their gradual temporal evolution, forms the basis for the speculation developed below, that three critical stages in the evolution of tectonic style on Earth may have occurred: (1) initially any thin crust formed was completely recycled by viscous drag forces associated with rapid convective circulation. (2) As these forces diminished, crust accumulated like scum, possibly to form a globe encircling layer. As such crust thickened by continuous outpouring of lava and tectonic

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compression, it would have been recycled by remelting at the base. Archean terrains have features compatible with this hypothesis. (3) Further decrease in heat flux and increase in negative buoyancy culminated in subduction of relatively thinner segments of this crustal scum, initiating plate-tectonics, and development of the present continent–ocean dichotomy. The essential details of this argument are as follows.

Continent–ocean dichotomy

Dissipation of up to 45% of the contemporary heat flux is attributed to the creation and cooling of new oceanic lithosphere[9]. The balance, produced by some combination of radioactive decay within the lithosphere (the upper continental crust in particular[11]) and a sublithosphere flux is conducted to the surface. (Although thought necessary to explain the observed heat flow values, the source of the flux is not clear. It could be associated with the small-scale convection of McKenzie and Weiss[8], or Richter and Parsons[12], or be generated by viscous dissipation associated with convective flow, as suggested by Schubert *et al.*[13].) The manner of dissipation of the significantly higher Precambrian heat flux[7,8] is becoming a focal point of the debate concerning Precambrian tectonic style[14–16] and whether or not plate tectonics were operating[17]. The more conventional uniformitarian view assumes that the mechanism involving the creation, cooling and subduction of new lithosphere, which accounts for 45% of today's flux, must have been at least proportionately more vigorous in the past[18]. In this view a greater length of spreading ridge, or faster spreading, is required to accommodate the higher flux. But this cannot be the case if negative buoyancy is the critical or dominant component of the plate driving force. Faster spreading would imply less time between ridge and trench, and greater ridge length would imply less distance between ridge and trench; in either case less cooling and hence less negative buoyancy could be achieved.

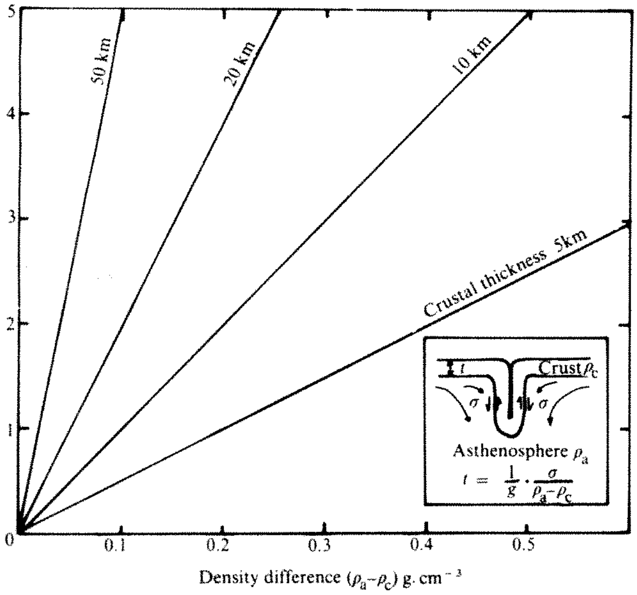

Fig. 1 Thickness of positively buoyant crust which would be in equilibrium with an asthenosphere-imposed downward-dragging shear stress (see inset). Curves for various crustal (or lithospheric) thicknesses are plotted as a function of shear stress and positive density difference ($\rho_{\text{crust}} > \rho_{\text{asthenosphere}}$). $t = (1/g)(\sigma/\bar{\rho}_a - \rho_c)$.

With respect to contemporary plate tectonics, buoyancy-powered subduction can occur as soon as the average density ($\bar{\rho}$) of the lithosphere is greater than the underlying asthenosphere. Basalt ($\rho = 3.0$)[19], by itself, is positively buoyant with respect to the asthenosphere ($\bar{\rho} \sim 3.22$); it is the growth of cold mantle lithosphere ($\bar{\rho} \sim 3.30$) which increases the average density and eventually renders the lithosphere negatively buoyant. If, as has been claimed[20], the residual periodotite from which the basalt was derived is also positively buoyant, then even more cooling and growth of lithosphere is required to achieve negative buoyancy.

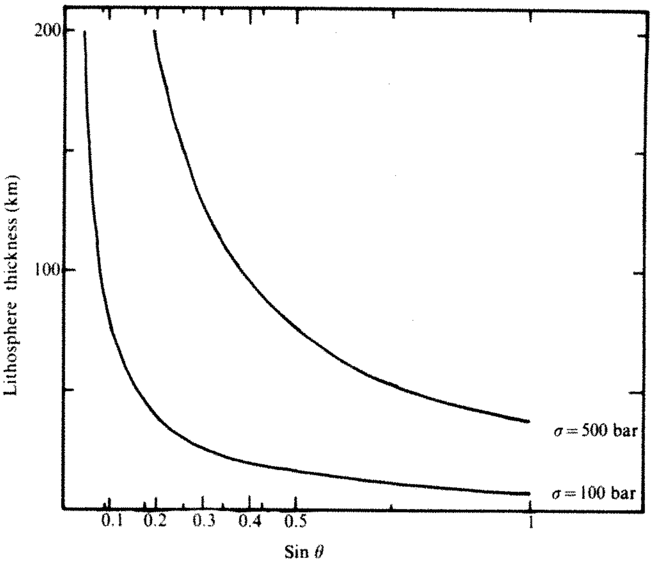

Fig. 2 Differential shear stress (σ) which would be generated along the Mohorovic discontinuity as a function of inclination or tilt (θ) of this boundary and total lithosphere thickness. Differential stress arises from positive buoyancy of crust relative to negative buoyancy of cold mantle part of the lithosphere. Model assumes lithosphere, isostatic with asthenosphere ($\rho_a = 3.22$) and consisting of 17.7% crust ($\rho_c = 2.85$) above mantle ($\rho_m = 3.30$). $t_{\text{lithos}} = 7.6\sigma/g \sin\theta$.

The average magnitude of the negative buoyancy required to trigger buoyancy-powered subduction may be approximated in terms of the average lithosphere subducting today which is about 80 Myr old, 75 km thick, and has a heat flow of ~60 mW m^{-2} (1.4 HFU)[21]. If twice this heat flow (120 mWm^{-2}, ≈lithosphere 20 Myr old, ~40 km thick) is taken as the maximum commensurate with buoyancy-powered subduction, then there was a time earlier in Earth history before which it was unlikely to occur. Even using the cooler (relative to a chondritic Earth) extrapolated heat flow curve of Wasserburg *et al.*[7], the average surface heat flow from the Earth was in excess of (120 mW m^{-2}) until ~1,500 Myr after the Earth formed[6]. On this basis subduction, powered primarily by negative buoyancy forces, could not have started until relatively late in Earth history.

Viscous drag

Although the lithosphere was likely to have been thinner when heat flow was higher, the viscous drag of more rapidly circulating asthenosphere would certainly have been stronger. Assuming that the Earth was hot enough for mantle-wide convection soon after its formation, McKenzie and Weiss[22] conjectured that originally this viscous drag force may have been as much as

Table 1 Correlation between tectonic sequence and Earth history

Tectonic sequence	Date (Myr BP)	Stages in Earth history
Viscous drag subduction	→ ~3.6 by	Complete cycling of crust
Scum—crust	3.6 → ~2.6 by	Archaean granite—greenstone terrains are remnants
Buoyancy powered subduction		
(1) Decoupling of mantle lithosphere from crust	2.6 → ~0.6 by	Cause of Proterozoic intracratonic mobile belts
(2) Complete lithosphere subduction	2.6 → 0	Growth and maintenance of ocean: continent crustal dichotomy

5×10^7 Nm^{-2} (500 bar). This, they pointed out, would have been more than sufficient to fracture the lithosphere or crust. At this time, however, the lithosphere may well have been so thin as to consist of little more than positively buoyant basaltic crust floating on asthenosphere. Being gravitationally stable, such a lithosphere would resist subduction, and it would thicken by overthrusting above the downwelling convective zone. It is possible, however, that the viscous pall of downwelling asthenosphere could effectively drag even positively buoyant thin crust (or depleted mantle lithosphere fragments) back into the mantle. The relationships among viscous drag force, density contrast and lithosphere thickness necessary for this to occur are shown in Fig. 1. Even though the model used is oversimplified, it shows, for example, that lithosphere slabs 10 km thick with an average density contrast of +0.25 g cm^{-3} could be dragged down by a shear stress of ~250 bar. On these terms, it can be claimed that the early Earth may have consisted of a mosaic of small, thin plates, circulating with the vigorously convecting asthenosphere beneath, a model similar to that proposed by McKenzie and Weiss[6].

As heat flow declined, the velocity of the circulating asthenosphere and the associated viscous drag force would presumably decrease. With slower spreading, however, the average age of the plates would increase, the mantle component of the lithosphere would become thicker and the average positive $\Delta\rho$ would decrease, eventually becoming negative. All the latter changes would make the lithosphere more subductible. Thus subduction driven by viscous drag forces may well have evolved continuously into buoyancy-powered subduction such as we have today.

Discussion

Two aspects of this simple evolution, however, merit further consideration. First, before the onset of buoyancy-powered subduction, a stage may have intervened when the viscous drag was insufficient to pull down positively buoyant lithosphere. As a result, crust may have accumulated like scum until, conceivably, the whole Earth was covered. Recycling of this crust would have occurred because thickening by tectonic compression and continued outpouring of lava on the surface would inevitably have led to remelting at the base. Eventually, however, the right combination of thin crust, relative to thick mantle lithosphere, necessary for critical negative buoyancy, would have been achieved somewhere on the globe, and buoyancy-powered subduction would have commenced. Complementary spreading would form protooceanic crust, and the growth of the ocean–continent crustal dichotomy could have begun. Second, cooling and thickening of mantle lithosphere beneath thicker crust could lead to their decoupling and independent 'subduction' of the former. Decoupling of lithosphere from crust would require that the shear stress on the Mohorovicic discontinuity exceed some critical value.

Such a differential shear stress could arise from the intrinsic positive buoyancy of crust relative to the negative buoyancy of the cold mantle lithosphere below. As long as the density gradient was perpendicular to the gravitational force (that is, the Moho was horizontal), however, there would be no differential shear stress. But if the discontinuity became tilted, a shear stress would be developed which might suffice for decoupling. Assuming that the average lithosphere was isostatic with respect to asthenosphere (that is, $\bar{\rho}_l = \rho_a$), then the relationship between lithosphere thickness and tilt of the Moho needed for decoupling, for two critical shear stresses, is shown in Fig. 2. With a 25-km thick crust, a tilt of 6° would cause a Moho shear stress of 100 bar; such a tilt would be associated with a warp of half amplitude 15 km and a half-wave length approaching 300 km. When heat flow was higher, the temperature at the Moho was higher, and the critical shear stress for decoupling was less. Decoupling by this means, therefore, seems feasible. Stresses associated with such independent subduction of mantle lithosphere slabs, decoupled from crust above, may have been responsible for the deformation in intracratonic mobile belts common in Proterozoic terrains[23]. Today, perhaps, with subcratonic Moho temperatures averaging 500° C, and thicker lithosphere, the tilts and shear stresses necessary for decoupling cannot be achieved.

The degree of validity of the simple relationships proposed here is not known. Even if they are pertinent, the temporal evolution of the magnitude of the parameters involved in the relationships is also unknown, but the trend with the inescapable decline in heat flux must be correct. As Table 1 shows, the appeal of the tectonic sequence postulated lies in the possible correlation with the observed major stages in Earth history.

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Injection events in ocean history

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The reconnection, with the world ocean, of temporarily isolated ocean basins results in injection either of hypersaline waters favouring abyssal stratification and stagnation, or of brackish waters from high latitudes leading to a low salinity surface layer. Mass extinctions and sudden climatic change are closely associated with Arctic injection.

SEVERAL major palaeoceanographic events have become well defined recently through the results of deep-sea drilling[1,2]. We propose here that several of these events are manifestations of the same type of occurrence, that is, the injection into the world ocean of saline or of fresh water from a temporarily isolated large basin. We first enumerate the events, and then consider the evidence for isolated basins and the timing of opening, the

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physical processes governing the injection, and the compatibility of our model with published data on isotopes and carbonate preservation. We also present the first detailed stable isotope stratigraphy across the Cretaceous–Tertiary boundary and interpret it in terms of the injection model.

Major events of the past 100 Myr

The phenomena to be explained are: (1) the mid-Cretaceous event or series of events (Aptian–Turonian) which produced worldwide changes in carbonate deposition[2] and which brought notable changes in marine biogeography, especially that of reefs, bivalves and nannofossils[3–6]; (2) the Cretaceous–Tertiary boundary event which brought about great changes in plankton assemblages[7–14]; (3) the Eocene–Oligocene event, which is marked by a sharp decline in diversity of plankton[15–17], a change of benthos[18], a pronounced lowering of the CCD[19,20] and by a sudden drop in the temperature of deep waters[21–23]; (4) the Miocene–Pliocene boundary which is marked by diversity decline of planktonic foraminifera[15,16] and by cooling[24] and Antarctic ice expansion[25], and which heralds the onset of northern glaciations[21,25,26].

Many explanations have been proposed for these events, especially for the Cretaceous–Tertiary boundary, which produced the most profound changes in evolutionary history during the past 200 Myr; these hypotheses are reviewed elsewhere[27,28]. Here we ask whether these events might be linked by a common oceanographic phenomenon: exchange with a previously isolated basin. Such an exchange was recently proposed for the Cretaceous–Tertiary boundary[29].

Recent palaeogeographic maps[30] show that preceding each major event a large isolated basin existed whose exchange with the world ocean was restricted to a more or less severe degree. In the Aptian, salt was deposited in a restricted proto-South Atlantic[31]. The same basin received highly carbonaceous sediments in late Aptian to Albian time[2,32], showing its restricted status. In the Maastrichtian, the Arctic Ocean was essentially isolated with both the Labrador Passage and the Greenland Passage closed[30,33]. In the Eocene, a similar situation prevailed, with the Labrador Passage and the Norwegian–Greenland seas closed[30]. In the early Miocene the western Tethys became isolated by closure of Africa and Eurasia[26] and in the late Miocene it went dry intermittently and accumulated large salt deposits[34–37].

In all four cases, the restricted basins seem to have opened for full exchange with the world ocean at about the time of crisis, and perhaps exactly coincident with it. Assuming that this is more than a coincidence, are there physical reasons that such an event should lead to worldwide biological and geochemical changes?

We think there are: the global heat budget requires polewards transport of latent heat, especially with warm climate in high latitudes[38]. Thus, each of the restricted basins is likely to be either more saline (proto-South Atlantic, Mediterranean) or fresher (Arctic) than the world ocean. A sudden onset of exchange, therefore, would upset the existing global density stratification, with profound effects on ecological stability and fertility of the ocean.

In principle, a strong influx of heavy (very saline and/or very cold) water will isolate abyssal waters from overlying intermediate and shallow waters, by increased density stratification. Likewise, a strong influx of fresh water or very warm water, will isolate the upper ocean layer from the waters at depth. However, the exact effect of a catastrophic influx depends on the existing stratification. If, for example, a widespread oxygen minimum exists, influx of heavy water can instantaneously lift the minimum towards shallower depths, provoking a global upwelling phenomenon (the surface water withdraws into the newly opened basin). Other sequences can be readily envisaged; for example, with intruding layers becoming sandwiched between existing ones and thus decreasing stability rather than increasing it. We are concerned here, however, with injection of waters of extreme density difference, which sink to the bottom or float on top of the ocean.

Cretaceous anoxic events

In the case of the mid-Cretaceous period of anoxic events[31,39,40] we postulate that the stability of the water column was greatly increased, first in the North Atlantic and later—and sporadically—in the entire Pacific by saline outflow to the north from the early South Atlantic starting in the Aptian. These brine injections reinforced haline stratification in an already salinity-layered world ocean with oxygen-poor and carbon dioxide-rich deep waters[2,41]. Deep water exchange between North and South Atlantic may have begun sometime in the Cenomanian, shallow-water exchange of normal salinity having been demonstrated for at least as early as the late Albian[42].

We envisage the mid-Cretaceous South Atlantic as being filled with heavy saline waters, to a level commensurate with the Walvis–Rio Grande sill. These saline waters were produced on surrounding partially flooded shelves and possibly also from the dissolution of pre-existing exposed Aptian salt deposits. The South Atlantic would have acted as a source of heavy bottom water for some considerable time, with pulsing supply, in response to climatic variation on the shelves. This pulsing would have translated into intermittent anaerobism, as reported by Lancelot, Seibold *et al.*[43]. Supply of nutrients and preservation of organic matter in the Atlantic were provided by estuarine circulation[44,45]. The South Atlantic surface and intermediate waters were estuarine with respect to the southern ocean, with intermediate cold waters moving north on top of the saline bottom layer and upwelling in the basin. This intermediate water brought the austral faunas described by Premoli Silva and Boersma[46]. The North Atlantic was estuarine with respect to the Pacific with intermediate cold waters moving east on top of the saline deep layer, and subsequently upwelling especially in the eastern Atlantic basin. Both a broad oxygen minimum and bottom-near oxygen deficit resulted, which lasted through Turonian time[2,47]. The oxygen minimum also reached into adjacent shelf sea areas, by estuarine shelf circulation, generating "tremendous areas of well laminated, dark organic rich shale"[48] deposited in conditions unfavourable for benthic foraminifera[49]. At the foot of the eastern continental slope, the abyssal saline layer favoured the precipitation of dolomite, and, through the accumulation of CO_2 of ankerite and siderite, as described from Site 138[50]. The mid-Cretaceous anoxic period ceased (for the deep sea) when the South Atlantic ceased to be restricted but became a throughway for austral cool deep waters. These waters were able to mix with the tropical saline inflow because of their similar densities.

Cretaceous termination

In the case of the Cretaceous termination[11,28], exchange with a previously isolated Arctic Ocean filled with fresh or very low salinity water has been postulated[29]. The isolation occurred during the latest Cretaceous regression. On tectonic opening of the Labrador Passage, the light water of the Arctic would be readily displaced by the surficial salt water of the North Atlantic. Assuming from palaeogeographic maps[30] and from present conditions that the volume of the Arctic is about 1.3% of that of the world ocean, and assuming complete isolation, we obtain a layer of fresh water some 53 m thick, as a maximum estimate. Mixing this layer to one-half the normal salinity makes a layer over 100 m thick. Hence it includes the entire photic zone. The salinity would be such that open ocean plankton (plus its trophic dependents and stenohaline shallow water benthos) could not survive, as observed[7,9,10,14,27,51]. Coastal plankton, being less sensitive to lowered salinity[52] and perhaps certain abyssal plankton, such as deep-living radiolarians, would be much less affected. Likewise, deep-living benthics would not be affected. Indeed, deep-living benthic foraminifera do not show unusual changes at the boundary (refs 53, 54 and L. Tjalsma, personal communication.). The ensuing short-term climatic changes would affect land animals and to a lesser degree land plants[35].

Once the unfit planktonic species had been eliminated, successive exchange events (from slight changes in sea level or in sill tectonics) produced intermittent Arctic injections and blooms of opportunistic survivors. Such blooms are well known from the earliest Tertiary, involving both thoracosphaeres and braarudosphaeres[13,14,56–60]. When the Labrador Passage had widened sufficiently[61], isolation of the Arctic ceased and so did injection of fresh water. Radiation of plankton species followed in the late Palaeocene[15–17].

Eocene termination

In the case of the Eocene–Oligocene boundary we again postulate exchange with a previously isolated Arctic, the isolation being produced by regression and by closing the Labrador Passage sometime during the Eocene[30]. This time the opening is through the Greenland Sea Passage[62]. Again, we assume the Arctic was fresh or had very low salinity, on the basis of the requirement that latent heat must be brought into this polar area in sufficient amounts to keep it ice free. The Eocene termination did not compare with the Cretaceous one in severity; we ascribe this to a more gradual water exchange, to the lack of a maximum exchange event, to a moderate brackishness of the Arctic at the end of the Eocene, or a combination of these. In other respects the two terminations are closely similar: open ocean forms do not survive and coastal forms flourish. We see small unspecialised planktonic foraminifera reminiscent of today's upwelling regions, and blooms of *Braarudosphaera* ('disaster forms' of Percival and Fischer[13]), as reported by Maxwell *et al.*[63] for the South Atlantic, and by Roth[64] for the Indian Ocean. In this view then the overall parallelism of plankton aspects of Palaeocene and Oligocene are due to the same cause.

The main difference between the two terminations (besides the amplitude) is the effect on benthic forms. At the Eocene–Oligocene boundary they are severely affected by the onset of production of cold bottom waters[18,21,23]. We ascribe this difference between the Maastrichtian and the Eocene termination to a feedback effect, which had negligible consequences in the first, but greatly increased snow cover (and possibly ice formation) in the Antarctic during the second. Our argument is derived from first principles of physical oceanography and climatology[65]. The introduction—presumably repeatedly—of a low salinity layer on top of the world ocean had the effect of temporarily obstructing the heat exchange between deep and shallow waters. As far as the heat budget of the Earth is concerned, such layering has the effect of reducing the ocean to a film of water hundreds of metres thick instead of thousands. Thus, the moderating influence of the ocean on climate is greatly reduced and seasonality increases correspondingly. The layering effect also increases the temperature of surface waters in the tropics, for the time of its duration. Both increased high latitude seasonality and increased tropical temperature favour transport of humidity to polar areas and its precipitation as snow. Once snow spreads, positive albedo feedback has a tendency to keep it there[66], resulting in a perennial polar high pressure cell with ensuing aridity.

In this view, then, the terminal Eocene injection event was the trigger for covering Antarctica with sufficient snow to move the polar front out to the shelf, which resulted in the production of cold bottom water. The cooling came at a time of regression, and albedo feedback through dry land areas[67] could be responsible for the overall drop in tropical temperatures in the Oligocene ocean[68]. We do not doubt the importance of the circumpolar current in this context[69] but merely argue that it is part of the setting, together with the polar position of Antarctica[70], and that the opening of Drake Passage (the timing of which is very uncertain) was not the trigger event. The botanical evidence indicates that vascular plants persisted on the Antarctic coast into the late Oligocene and that complete coverage by ice did not occur before Miocene time[71,72].

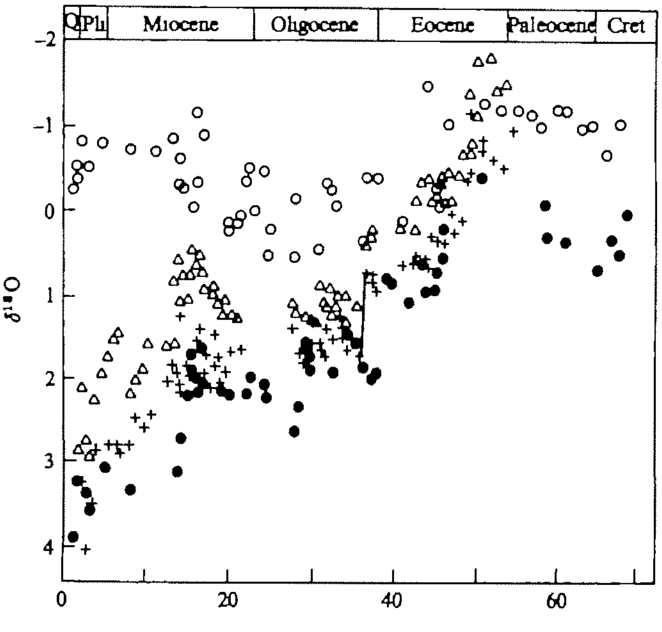
Messinian event

Isolation of the Mediterranean started in the early Miocene at its eastern end and culminated in the Messinian evaporite event[33–36,73–75]. We note the great amount of salt estimated to have been deposited in the basin during the latest Miocene (6% of ocean total)[37]. Many inundations were necessary to advect this salt[76] and intermittent water exchange with the world ocean probably occurred. In this view, the Mediterranean acted as a pulsating injector of saline waters, much like the South Atlantic some 100 Myr earlier. Again, stability of the world ocean was increased intermittently, with the described effect on seasonality, cooling and ice formation. An unusual cooling in high southern latitudes is shown for Site 284 (ref. 24) at the appropriate time, and the formation of ice strongly increased, according to the oxygen isotope curve from Site 281 (ref. 21). A temperature peak at the end of the Miocene is suggested for mid latitudes in the north-east Pacific[77]. The co-occurrence of warming in the subtropics and cooling in high latitudes would agree with our hypothesis. We do not doubt the importance of the correct setting for increasing the formation of ice, including the closing of the Panama Passage[78,79], but stress the role of stratification as a trigger of positive-feedback mechanisms.

Oxygen isotope stratigraphy

If stable stratification is associated with the events under discussion, and if it has the effects postulated, we should see warming in low latitudes and increased seasonality in high latitudes. Unfortunately, our hypothesis cannot be tested using the available information. The following statements illustrate the type of analysis necessary for evaluating the reality of injection events, and their ramifications (Fig. 1): (1) in the published records there is no substantial change of oxygen isotope values between the late Cretaceous and Palaeocene, although a minor temperature fall across the Cretaceous–Tertiary boundary seems to be indicated[80,81]. Thus, there is no evidence that the termination event acted as a trigger within a setting favouring change. Average temperatures were

Fig. 1 Oxygen isotope values of Cainozoic foraminifera (after Savin[68]). Data mainly from Douglas and Savin[81] and Shackleton and Kennett[25]. The short straight line at the Eocene–Oligocene boundary indicates the position of the drastic isotopic temperature drop (see ref. 23). Central North Pacific: ○, planktonic; ●, benthic; Southern Ocean: △, planktonic; +, benthic.



apparently fairly similar before and after the event. (2) The oxygen isotope values for early Eocene planktonic foraminifera of Site 277, south of New Zealand are the same or lighter than those from Site 47 on Shatsky Rise then located in the subtropics. For a 5 °C difference, for instance, between subtropic and subantarctic temperatures these southern values are too low by at least 1‰. We ascribe this difference to evaporation–precipitation effects[82], and postulate a difference in salinity of at least 4‰. Rainforests existed in Eocene time in southern Australia and in the Ross Sea area[72]. This supports our deduction from heat budget considerations of high rainfall in high latitudes. (3) The $\delta^{18}O$ values of low and high latitude foraminifera (and benthics) begin to separate in the mid-Eocene. This indicates that the global temperature gradient increased and supports our contention that the Eocene termination was a trigger event, accelerating the existing trend. (4) There is no indication of a sharp lowering of tropical temperatures at the very end of the Eocene. Our hypothesis would not favour such a sharp lowering (instead it calls for climatic instability). (5) The drastic mid-Miocene drop of high latitude isotope values, due to an unknown combination of making Antarctic ice[21] and of further cooling[68], occurs concomitantly with high tropical temperatures. Although we do not postulate injection for this event, we see the marked transgression at the time[83,84] as an event simulating injection of light surface water. The transgression leads to the formation of a warm water layer, by the additional capture of solar radiation on the flooded shelves.

We have not shown the available oxygen isotopes from the mid-Cretaceous as they are still too scarce for the type of analysis presented. Nannofossil data from the North Pacific[81] show maximum temperatures near the Albian–Cenomanian transition, as predicted by our hypothesis. The same is apparently indicated by data from European belemnites, although various interpretations are possible[85].

Carbon isotope stratigraphy

The data for carbon isotope stratigraphy have been summarised by Fischer and Arthur[60]. We agree with their conclusion that periods of strong oxygen minima correlate with the deposition of organic carbon which is enriched in ^{12}C (ref. 86) and with depletion of ^{12}C in carbonates[12,83]. However, over short timescales, we postulate that the oxygen minimum produced by an injection event will tend to enrich the plankton shells in ^{12}C (ref. 87).

In agreement with this expectation, we note marked enrichment with ^{12}C in the planktonic foraminifera just before the Eocene termination, in the data given by Shackleton and Kennett[21]. Also, a slight warming is indicated in the detailed oxygen isotopic curve of Kennett and Shackleton[23], just before the event, which again agrees with the concept of increased stratification as the trigger. Likewise, there is a remarkable enrichment in ^{12}C of *Uvigerina* from Site 284, at the very end of the Miocene[25]. We interpret this to indicate stable stratification and oxygen depletion with concomitant increase of dissolved ^{12}C, from combustion of organic matter. The stable stratification, by our hypothesis, is provided through Mediterranean injection.

Preservation stratigraphy

Injection events should produce a preservation signal, as they would change mixing rates of the ocean, and hence fertility of the photic zone and affect the p_{CO_2} of deep waters. As in the interpretation of all other signals, caution is necessary to separate effects of a trigger event from those of the change in setting which ensued.

For the Cretaceous–Tertiary boundary widespread dissolution has been proposed[12,88]. This idea is based on evidence from shallow sections, and may be correct for the upper water layers. In the sections at Caravaca (south-east Spain), Zumaya (Gulf of Biscay) and at Stevn's Clint (Denmark), for example, there is a dearth of carbonate in a thin boundary layer of clayey sediment deposited in anaerobic conditions[13,54,89–90]. A widespread oxygen minimum developing underneath a low salinity lid could produce such a signal. In the deep Atlantic, the transition is not marked by a dissolution event. In contrast, at Sites 356 and 384, for example, the foraminifera and nannoliths in the basal Tertiary (*G. eugubina* Zone) show excellent preservation[52,91–93]. At site 358 there is a preservation climax in the lowermost Palaeocene[91].

The Eocene–Oligocene event is marked by a sudden drop of the CCD from high, ridge crest elevation to abyssal depths[19,94–96] and in the ratio of planktonic–benthic forams[97] which can be interpreted as indicating improved preservation, and also decreased productivity[98].

Another clue to the type of signal one might expect is given by the last deglaciation, 14,000–9,000 yr ago, when large amounts of meltwater were injected into the global ocean which apparently resulted in stable stratification of surface waters[99]. This event was accompanied by a preservation spike which ended abruptly in a dissolution pulse[100]. A simple explanation of this sequence would be that surface fertility was lowered during maximum stratification, letting the deep waters move towards chemical equilibrium, and that dissolution started when productivity resumed in upper waters and supplied organic carbon to still stagnating deep waters. However, the interpretation of the deglacial preservation sequence is complicated by re-deposition of shallow water sediments at the end of the glacial and by Holocene carbonate build-up on the flooded shelves[101].

We believe that all three preservation spikes can be explained by a fertility crisis in the photic zone and ensuing saturation of bottom waters. In the case of the Maastrichtian–Danian boundary event excessively stable surface stratification may have prevented reflux of nutrients, and a widespread oxygen minimum might have destroyed available nitrogen[102–103]. The decay of stratification after the injection at the Eocene–Oligocene boundary was accelerated by the progressive replacement of haline circulation by thermal circulation[83].

For the mid-Cretaceous event, the evidence indicates a worldwide rapid shallowing of the CCD in the Aptian, a temporary lowering in the Albian–Cenomanian, followed by another dissolution pulse in the Turonian[2]. The variation in carbonate deposition is closely matched by enrichments in organic carbon in deep-sea sediments, which parallels a general change from low oxygen content (carbonaceous sediment) to ventilation (oxidised sediment)[60].

The mid to late Miocene dissolution climax[104,105] coincides with the beginning of isolation of the western Tethys, as well as a marked transgression[83,84]. Of special interest is a dissolution pulse across the Miocene–Pliocene boundary, resulting in a high concentration of the genus *Sphaeroidinellopsis*[91,106]. We believe this is due to an increase in deep-sea p_{CO_2} resulting from haline injection. This dissolution pulse is, incidentally, superimposed on a general trend of a falling CCD and improvement in preservation, showing that the terminal Messinian event does not produce the trend, although it may have accelerated the change at the boundary. In Core RC 12–66, from the east equatorial Pacific, maximum contrast of high and low carbonate values occurs at the end of Magnetic Epoch 5, near the boundary[107]. The dissolution event in the earliest Pliocene seems to be the most severe one over several million years (event X in Kaneps[108]). According to our hypothesis, this event in the Pacific and the *Sphaeroidinellopsis* zone in the Atlantic realm are both expressions of an increase in p_{CO_2} due to stable stratification.

The available information on isotopic stratigraphy and on preservation stratigraphy is compatible with our injection–stratification hypothesis. We now return to the major event of the past 100 Myr, the Cretaceous–Tertiary boundary, to test further the compatibility of hypothesis and observation.

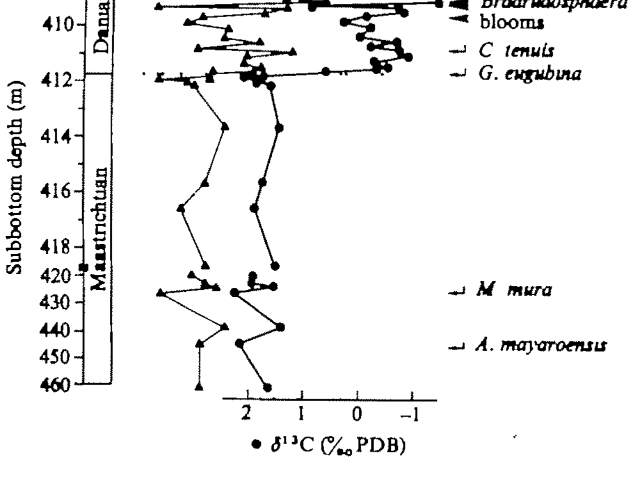
Cretaceous termination at site 356

Recent successes in drilling the deep-sea contact of the Cretaceous–Tertiary boundary[109,110] have made it possible to study in detail this major event in ocean history[14]. Here we present the first detailed stable isotope stratigraphy across the boundary. closely dated with coccoliths. Procedures and supporting material will be reported in more detail by H.R.T. *et al.*

The oxygen isotope and carbon isotope determinations shown (Fig. 2) were done on the fine fraction (<62 μm). We believe the record is continuous or nearly so, because it correlates well with that from Site 384, which has also been dated biostratigraphically in great detail[14]. In both sections the evolution of early Danian assemblages is characterised by an initial bloom of *Thoracosphaera* spp., with *Markalius astroporus*, *Zygodiscus sigmoides* and *Cruciplacolithus* spp. present, and by a subsequent bloom of *Braarudosphaera* spp. and dominance of *Cruciplacolithus* spp. In both sections the latest Cretaceous and particularly the earliest Danian nannoliths are well preserved[56,61]. It has been shown that in well preserved samples the stable isotopic variation of the nannofossil fraction parallels that of the planktonic foraminifera[111,114]. At Site 356 the uppermost Cretaceous oxygen and carbon isotope values are near −2.0‰ and +1.9‰ respectively, which are typical for Maastrichtian plankton elsewhere in low latitudes[81]. At the termination event both δ^{18}O and δ^{13}C values drop sharply within 40 cm. 'Warming' and the formation of a strong oxygen minimum are apparently indicated. The Danian coccolith flora is dominated by thoracosphaerids and baarudosphaerids, neither of which, however, exceeds 25% of the total fine fraction in any sample.

How can the sudden warming across the Cretaceous–Tertiary boundary be produced? In principle, a large amount of heat could conceivably be supplied from space (for example, by a solar flare) or from Earth's interior (for example, large outpourings of submarine lava). We find both hypotheses unattractive, although the evidence may be insufficient to reject them outright. According to the injection hypothesis, the warming has a strong signal of fresh water composition in it, which makes it unnecessary to search for an extraneous heat source. Lowered salinity readily explains both the demise of the tropical late Cretaceous forms and the bloom of typically coastal braarudosphaerids. The magnitude of the fresh water signal can be estimated very roughly. Assuming that the precipitation of water in high latitudes in the latest Cretaceous was tagged with the isotopic signal of today's mid-latitudes in the rainbelt[82] we obtain, say, $\delta^{18}O = -7‰$ for the fresh water injected from the Arctic. We take the average seawater δ^{18}O value as 0‰, for simplicity, and the salinity as 35‰. This yields a δ^{18}O of 0.2‰ for each 1‰ salinity change, when mixing fresh and saltwater. The excursion of δ^{18}O towards low values at termination time is −2.0‰, which translates into a lowering of salinity by 10‰, to 25‰, if the entire signal is due to mixing with fresh water. To the degree that warming occurs in the postulated stable surface layer, the salinity difference between normal and diluted seawater would be lessened. However, the difference must be increased to the degree that benthic mixing and the admixture of laterally transported Cretaceous nannoliths reduced the amplitude of the isotopic signal. We think that the estimate of a surface water salinity of 25‰ during the time of crisis is reasonable.

Fig. 2 Stable isotope curves of the fine fraction carbonate for DSDP Site 356, Sao Paolo Plateau, South Atlantic (analysis by John S. Killingley).



With the δ^{13}C signal, two effects must be considered. First, a marked decrease of plankton productivity would decrease the production of ^{12}C rich solids such as faecal pellets. The lack of removal of organics from surface waters would then decrease the carbon fractionation. Second, strong surficial stratification would result in the formation of a shallow, strong oxygen minimum. The effect of subsurface anaerobism on the δ^{13}C value in water can be estimated[112–114]. The relationship between the carbon isotopic composition of total dissolved carbon dioxide and the apparent oxygen utilisation (as calculated from Williams *et al.* (Fig. 2)[113]) is approximately $\Delta\delta^{13}C = -AOU/133$, where $\Delta\delta^{13}$C is in ‰ PDB − 1 and AOU is in μmol l^{-1}. For an initial content of about 200 μmol of oxygen l^{-1} in warm surface waters of normal salinity[115] and for total consumption, we get a signal of −1.5‰ in the δ^{13}C, all other factors being equal. This signal is too small to explain the observed change which is also muted by the considerable reworking of Cretaceous taxa. The injection hypothesis allows for a higher initial oxygen content due to reduced salinity and cooler (Arctic) water, by about 20–30%. Thus, a signal of about −2.0‰ is justified under the hypothesis. In addition, the demonstrated deviation from equilibrium of calcereous nannoliths towards lower $^{13}C/^{12}C$ ratios[114] may have been increased during post-termination time, due to sporadic bloom conditions, suggested by high abundances of thoracosphaerids and braarudosphaerids.

The rapid fluctuations in isotopic values in post-termination time seem to be real despite the somewhat wider sample spacing in the Upper Cretaceous. In part, such fluctuations can be produced by inhomogeneous benthic mixing, which has been observed at this boundary[109]. However, mixing can only scramble the frequency, and decrease but never increase the amplitudes of the signal. Thus, the impression remains that large fluctuations characterised post-termination time. This is also indicated by the sporadic blooms of *Thoracosphaera* ssp. and *Braarudosphaera* ssp.

At least two mechanisms exist for producing the fluctuations in the framework of the injection hypothesis: (1) repeated isolation and opening of the Arctic which led to repeated injection of freshwater; (2) climatic oscillations involving alteration of strong cooling and warming. Climatic oscillations could come from the effects of intermittent stratification on the CO_2 exchange of the ocean and the atmosphere[116]. Such oscillations together with decreased climatic equability would also have a profound effect on the survival of land animals[117]. It is interesting, in this context, to contemplate the extinctions of large land animals at the last injection event, the deglaciation some 12,000 yr ago[118].

We think that intermittent rapid addition to the world ocean of waters of unusual density produced in temporarily isolated basins in high and low latitude settings may account for some of the most puzzling oceanographic events discovered by deep-sea drilling, events which left their marks as mass-extinctions and widespread black shale deposits. Our hypothesis has implications for all Phanerozoic evolution, including the major mass extinction at the Permian–Triassic boundary.

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Do present levels of air pollution outdoors affect respiratory health?

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A sensitive lung function test does not show differences due to air pollution between lifetime residents in a rural area and those in a small industrial town in Connecticut. Also, there is no evidence that higher air pollutant concentrations elsewhere have any marked effects on the lungs. Severe pollution is dangerous and must be avoided, but at present, air pollution control outdoors does not deserve priority as a means of preventing chronic lung diseases.

WE have assessed the impact of urban residence on respiratory health in the population of an industrial US town, in comparison with a rural population which included lifetime dwellers as well as migrants who had previously lived in cities. We determined differences in respiratory health between these populations from (1) the prevalence of common respiratory symptoms, and of bronchitis and asthma, and (2) lung function tests which reflect airway calibre. As air pollution outdoors is one 'urban factor' which may affect the health of people living in cities, we monitored several air pollutants at different sites in both towns.

The urban area had a record of high air pollution (by US standards) in the recent past, but the urban–rural pollution contrast at the time of our study was modest. We therefore also compared our results with data on people living in more severely polluted urban areas, and in other rural areas, so as to expand the pollution contrast.

To assess respiratory health in both towns, we used a questionnaire and a sensitive, standardised lung function test. We studied children and adults of both sexes, and healthy persons as well as those with respiratory symptoms or disease. Our population samples were large and representative of the total population of both areas. In analysing the results, we took into account sex, race, age, body height, body weight, smoking habits, occupation and previous residence. We believe that this is the first report of a study which combines all these features, and that it allows more reliable conclusions concerning urban factors and chronic respiratory disease (other than lung cancer) than do previous studies.

Populations and air pollution

The urban site, Ansonia, Connecticut, is an industrial town where mean annual particulate concentrations were among the highest measured in Connecticut during 1966–72 (88–152 $\mu g\ m^{-3}$; ref. 1); high SO_2 concentrations (by US standards) were probably also common in the past. In 1973, particulate concentrations were lower but still significantly higher than at the contrasting rural site, Lebanon, Connecticut. Lebanon is a sparsely populated town without factories or major highways, away from cities. Outdoor pollutants (Table 1) were monitored in both towns for more than 1 yr, including the period of our population surveys[1,2]. Concentrations of total suspended particulates (TSP; high-volume samplers) and of nitrogen dioxide were significantly higher at the urban sites, as were nitrates and sulphates (data in refs 1, 2). There were no significant differences for sulphur dioxide and ozone.

In two geographically defined areas (the town of Lebanon and the 4th Ward of Ansonia), we attempted to study all residents aged ≥ 7 yr. Response rates varied from 91–96% among boys and girls (7–14 yr) in both towns to 56% and 80% among 25–64-yr-old adults in Ansonia and Lebanon, respectively. From a private census and interviews of nonresponders[3], we concluded that the responders adequately represent the total populations. There were only 20 black residents of Lebanon; our main analyses are therefore limited to the white residents of Lebanon and Ansonia (Table 2). Of the Lebanon subjects, 41% had previously lived in urban areas; possible selection factors made it important to consider these subjects separately from lifetime rural (LR) dwellers. The few 'previous rural' (PR) Ansonia residents were excluded from analysis. Three smoking categories were considered: lifetime nonsmokers, ex-smokers of cigarettes, and current cigarette smokers (Table 2).

Questionnaire

We used an extended version of the MRC bronchitis questionnaire (for text, see ref. 4). Questions were prompted by computer and read by trained interviewers; answers were recorded in computer memory. There has thus been no omission or loss of data. Detailed written instruction and supervision of interviewers by the investigators promoted consistency in the interviews. We were unable to allocate subjects at random to interviewers, but an examination of symptom prevalences by interviewer showed that interviewer variation had no important effects on our results. Previous urban (PU) and lifetime rural residents of Lebanon were seen by the same interviewers; interviewer variation can be excluded as a cause of differences between these groups.

Chronic bronchitis

First, we examined the symptom complex of chronic bronchitis (usual cough and phlegm, more than 3 months per year for ≥ 2 yr). In groups of men and women by age (25+ yr) and smoking habits, smokers always had higher prevalences than nonsmokers or ex-smokers, but urban–rural differences were absent in all groups. For example, 19.0% of the rural male adult current smokers had chronic bronchitis, compared with 17.0% of their urban counterparts. Among both urban and rural residents aged ≥ 45 yr, the prevalence of chronic bronchitis was less than half that found (with identical methods) among active and retired textile workers at risk from occupational cotton dust exposure[5].

Asthma

A history of bronchial asthma ('yes' to the question: 'have you ever had bronchial asthma?') was more common among rural than among urban residents, regardless of age. The difference was highly significant for males (6.7% in 1,142 rural (=LR+PU) males; 2.6% in 458 urban males; $\chi^2 = 9.99$, $P < 0.01$) and similar differences, although not significant, persisted when only lifetime urban and rural residents were compared and when smoking was excluded by comparing only nonsmokers. Smoking habits were not significantly related to a history of bronchial asthma (χ^2 analysis).

Cough, phlegm and other symptoms

Chronic bronchitis may be an insensitive index of urban–rural differences because it is uncommon among nonsmokers. For its component symptoms, as well as wheezing and dyspnoea, we examined the relative importance of the residence variable and of sex, age and smoking habits in a weighted-least-squares analysis[6] of all data in 15–64-yr-old nonsmokers and current smokers. We omitted children, because few of them had any symptoms, and the elderly, because they were too few in number. We also excluded ex-smokers; their symptom prevalences were usually close to those of nonsmokers. Table 3 summarises the best-fitting models. Cigarette smoking was the only variable consistently associated with increased prevalence of all symptoms, at $P < 0.001$. For usual cough and phlegm, a linear residence variable (that is, LU > PU > LR) was highly significant among nonsmokers but not among smokers. The linear residence variable for dyspnoea was complicated by interactions between sex and smoking and between LU residence and smoking. The association between residence and dyspnoea was most pronounced among nonsmoking women (that is, 12.8% in 211 LR compared with 19.2% in 151 LU women). Among nonsmokers (men and women), LR residents had the lowest dyspnoea prevalence; among smoking men and

Table 1 The two towns and their air quality

	Lebanon (Rural)	Ansonia (Urban)
No. of inhabitants	3,800	21,200
No. of inhabitants per km^2	29	1,178
No. of dwellings per km^2	12	248
No. of vehicles per km^2	26	650
No. of commercial buildings and factories per km^2	0.03	17.8
Sulphur dioxide ($\mu g\ m^{-3}$)	10.9 ± 1.6 (41)	13.5 ± 1.7 (50)
Total suspended particulates ($\mu g\ m^{-3}$)	39.5 ± 4.2 (44)	63.1 ± 3.7 (50)
Nitrogen dioxide ($\mu g\ m^{-3}$)	55.5 ± 6.1 (41)	87.8 ± 4.9 (50)
Ozone ($\mu g\ m^{-3}$)	84.7 ± 4.4 (28)	88.5 ± 3.3 (35)

Demographic and geographical data from 1970 US Census, US Geological Survey maps, and municipal registries. Air pollutant data were obtained at 3–5 sites in Lebanon and at 4 sites in Ansonia, from January to December 1973, and are means ± s.e.m. of 24-h average concentrations, except for ozone values, which are means ± s.e.m. of peak 1-h concentrations (no. of observations in parentheses). Pollutant measurements (for methods and details, see refs 1, 2) were made with supervised, accurately calibrated samplers placed 120 cm above ground level, at least 16 m from any road and at least 11 m from physical obstructions (such as homes, trees). TSP and NO_2 were significantly ($P < 0.001$) higher in Ansonia than in Lebanon; SO_2 and O_3 did not differ significantly.

Table 2 Population groups by sex, age, residence and smoking habits

Residence		Age (yr)					Smoking			Total
Males		7–14	15–24	25–44	45–64	65+	NS	XS	S	
Lifetime rural (LR)		232	116	152	96	18	342	122	150	614
Previous urban (PU)		117	49	135	72	20	165	90	138	393
Lifetime urban (LU)		81	81	71	74	17	144	77	103	324
Previous rural (PR)		2	6	9	10	5	14	9	9	32
	Totals:	432	252	367	252	60	665	298	400	1,363
Females										
Lifetime rural (LR)		228	133	195	107	23	456	85	145	686
Previous urban (PU)		112	84	200	95	21	274	78	160	512
Lifetime urban (LU)		101	96	95	136	26	275	59	120	454
Previous rural (PR)		4	6	13	14	4	29	7	5	41
	Totals:	445	319	503	352	74	1,034	229	430	1,693

LR, lifetime residence in Lebanon and other rural areas; PU, current Lebanon rural resident but past residence in urban areas; LU and PR, lifetime urban and previous rural residents of Ansonia Total number of subjects (3,056) represents the total studied (3,387) minus 331 excluded for one or more of three reasons: (1) smokers of pipes or cigars only; (2) 1 yr or longer work in dusty occupations (mines, quarries, foundries, potteries, cotton mills, asbestos factories); (3) unclear history of previous residence (urban or rural) Lifetime nonsmokers (NS) had never smoked tobacco in any form; additional smoking of pipes or cigars by ex-smokers (XS) or current cigarette smokers (S) was not considered. Among children (7–14 yr) the number of ex-smokers and smokers was too small for analysis.

women the PU residents had the highest prevalence (30.8% compared with 21.5% in both LR and LU residents). Residence effects were less significant or absent for wheezing, and they were complicated by interactions with age. The significant age variables in Table 3 reflect higher prevalences among older than younger subjects, except for usual cough, where the 25–44-yr-olds had more symptoms than those who were younger or older. Sex was significant for usual phlegm, being more common among men, and for dyspnoea, which was more prevalent among women.

Figure 1 shows the observed prevalences of usual phlegm in relation to all variables which contribute significantly to its prevalence. There are no interactions, and the fit of the model is the best of all five in Table 3. Observed prevalences among women are similar to those predicted by the model. Among young urban male nonsmokers, the model seems to overpredict prevalence, but the difference between actual and predicted prevalence is not significant. All smokers, regardless of residence, have a higher prevalence of usual phlegm than do LU nonsmokers.

Lung function

We selected the maximum expiratory flow volume (MEFV) curve as a simple, sensitive and comprehensive lung function test[4]. It provides information on lung volume (forced expiratory vital capacity, FVC) as well as maximum expiratory flow rates. In previous studies, maximum flow at mid-vital capacity (MEF 50%) detected airway-constrictor effects of cigarette smoke[7], textile dust[8] and air pollutants[9] better than did measurements such as peak flow or forced expiratory volume in 1 s ($FEV_{1.0}$). We recorded MEFV curves on-line with a computer[10], calibrated every 2 h with a standard curve delivered by a mechanical device[11]. From data on healthy lifetime nonsmokers (by race, sex and age group) in three US communities, including Lebanon and Ansonia, we have derived regression equations for FVC, $FEV_{1.0}$, the $FEV_{1.0}$/FVC ratio, peak expiratory flow rate (PEF) and maximum flow rates at two other points on the MEFV curve (MEF50% and MEF25%), as a function of age, height and weight[12]. In the present study, these equations served to account for effects of age, height and weight in comparisons of lung

Table 3 Weighted least squares analysis of symptom prevalences

Symptom	Variables				Interactions			Fit of model	
	Smoking	Residence	Age	Sex	Age with	Smoking with.	LU residence with:	*P*	% Variation explained
Cough	+++	+++*	++	0	Sex +++			0.719	74.8
Phlegm	+++	+++*	+	+++				0.998	81.1
Recent wheeze	+++	+†	+	0	Smoking ++		Age +	0.759	79.7
Frequent wheeze	+++	0	0	0	Residence ++			0.962	67.4
Dyspnoea 1+	+++	+++‡	++	+++		Sex ++	Smoking +++	0.910	77.7

The table includes variables and interactions which contributed significantly to the explanation of the variation in symptom prevalence among all lifetime nonsmokers (NS) and current smokers (S) in Lebanon and Ansonia (age 15–64 yr). Logistic models always gave a better fit than did additive models. If x is the proportion of subjects with symptoms, the logistic model uses a logarithmic transformation of x, that is, $\ln[x/(1-x)]$, as the sum of effects of smoking, residence, age, sex and their interaction. Definition of symptoms: Cough and phlegm on most days for at least 3 months per year; recent wheeze, wheeze within past 12 months; frequent wheeze, wheeze at least a few times each week; dyspnoea 1+, dyspnoea when hurrying on level ground or walking uphill, or worse. Significance of variables and interactions· +++, $P\leqslant 0.001$; ++, $0.001< P\leqslant 0.01$; +, $0.01< P\leqslant 0.05$; 0, not significant. Explanation of interactions: Age × sex: difference between sexes (M > F) increases with age. Age × smoking: age increase of prevalence greater for NS than S. Age × residence: 25–64-yr-old PU residents more wheeze than LR and LU; 15–24-yr-olds lower prevalence regardless of residence. Smoking × sex: less effect of smoking in females than males; NS females have relatively high prevalence. LU residence × age: effect of residence on prevalence increase more pronounced in 25–44-yr-olds than in 15–24- and 45–64-yr-olds. LU residence × smoking· LU smokers have lower prevalence than LR and PU smokers; LU nonsmokers have higher prevalence than LR and PU nonsmokers.

* Linear residence variable significant within NS only.

† LR < PU = LU, $P = 0.045$.

‡ Linear residence variable significant for NS and S.

function between groups of subjects. These comparisons were based on calculations of lung function residuals, that is, the differences between observed and predicted values of each measurement.

Analysis of variance of lung function residuals (Fig. 2) among LR, PU and LU residents by sex, age and smoking habits showed no significant differences for any of the measurements. LR, PU and LU nonsmokers had similar residuals, none of which differed significantly from zero or from each other. Smoking adults, on the other hand, had significantly more negative residuals than comparable nonsmokers, regardless of residence. Inclusion of adults with occupational exposure hazards (see legend to Table 1 for definitions) gave results similar to those in the population which excluded these persons.

Susceptible groups

Urban air pollution might not affect all residents, but only unusually sensitive groups within the urban population. Our data suggest that, if such groups exist, they cannot be readily identified on the basis of age (in those aged ≥ 7 yr), sex, race or smoking habits. Attempts to identify susceptible subgroups from questionnaire data were equally unsuccessful. Among smokers, the amount smoked seemed to be the main variable, affecting both symptom prevalence and lung function (unpublished observations), and there were too few nonsmoking urban (LU) residents who reported a history of asthma to allow a comparison of the severity of asthma among urban and rural residents. Among residents with usual cough and phlegm, lung function losses were minimal and not significant either among rural (LR) or urban (LU) residents. Thus, although there may be small groups of persons sensitive to factors in the urban environment that do not affect most people, we have not been able to identify them.

The 'urban factor'

Studies in several US cities[13] have linked excess chronic bronchitis among smokers and nonsmokers to sulphur oxide and particulate pollution. In contrast, we have found that only lesser degrees of certain symptoms, not the composite syndrome of chronic bronchitis, may be associated with urban air pollution, and that this association only occurs in nonsmoking adults (age 15–64 yr; Table 3, Fig. 1). Among smokers, the influence of smoking overrides any differences associated with residence. However, the differences in symptom prevalences between urban and rural nonsmokers are not accompanied by differences in lung function. Even flows on MEFV curves (MEF50%; MEF25%), which are sensitive indices of airway obstruction[14], did not differ. Thus, we have no objective evidence for substantial differences in respiratory health between urban and rural residents.

Table 4 Lung function in nonsmoking California and Connecticut residents

	Los Angeles	San Diego	Connecticut		
			LU	PU	LR
O_3 ($\mu g\ m^{-3}$)	~300	~150	~100	~100	~100
TSP ($\mu g\ m^{-3}$)	124	78	65	42	42
No. subjects	90	135	73	41	56
Height (cm)	162	163	158	157	160
$FEV_{1.0}$ (l)	2.44	2.43	2.10	2.08	2.25
MEF50% ($l\ s^{-1}$)	3.38	3.37	2.96	2.81	3.09

Data for white, female, lifetime nonsmokers aged 45–64 yr. Los Angeles and San Diego data from Cohen *et al.*[28]. Data for males are similar. Higher lung function values in nonsmoking California women are explained by their height. All function values are close to those predicted for healthy nonsmoking adult white females[12].

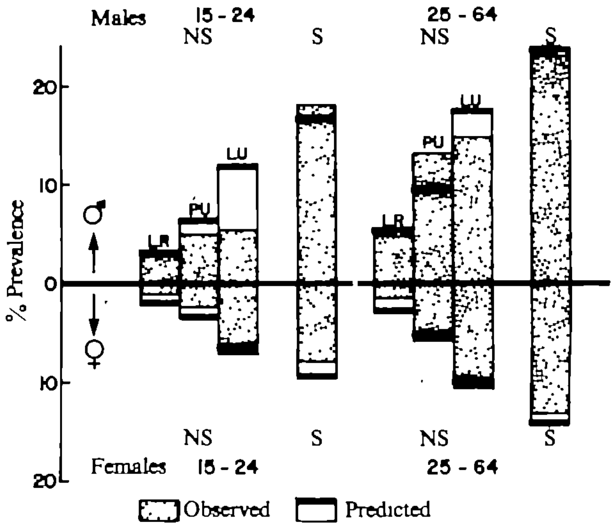
In the absence of lung function changes, what meaning should be attributed to the higher symptom prevalences among urban and previous urban nonsmokers? They might be due to trivial variables such as a different perception of questions by city and country people. The greater prevalence of dyspnoea among urban (LU) than rural (LR) nonsmoking women might be related to body weight (on average 4.1 kg more in 25–64-yr-old LU women than LR women). However, the LU–PU–LR gradient in prevalence of cough and phlegm among nonsmokers might reflect slight differences in respiratory health. For example, hypersecretion of bronchial mucus (leading to cough and phlegm production) may be more common among urban than rural nonsmokers. This disorder need not lead to progressive lung function loss[15]. It may be part of an adaptive mechanism (for example, increased mucus production may aid the clearance of pollutants) or it might represent incipient illness. Sputum from people in a polluted urban area may contain increased numbers of phagocytes and white cells[16]; these might secrete proteolytic enzymes which damage alveolar tissue[17], or they may protect lung tissue against inhaled particles, or both. No firm conclusion is possible, but the lack of function loss among our older symptomatic, lifetime nonsmoking urbanites suggests that the increased symptom prevalences are not associated with significant long-term deleterious effects on the lungs. Nor does our study indicate additive or synergistic effects between air pollution and smoking: lung function loss among smokers was similarly regardless of residence.

Our study cannot distinguish between air pollution and other urban factors as possible causes of the increased symptom prevalences among PU and LU nonsmokers. However, in a study designed to detect effects of air pollution peaks on daily symptoms among women in high- and low-pollution areas near Rotterdam, Wever[18] found significant associations, mostly in nonsmokers, of pollution peaks with cough, phlegm and dyspnoea, but rarely with wheezing. The similarity of Wever's findings to our own supports the assumption that in Ansonia, too, air pollution may be the 'urban factor' responsible for increased symptoms in lifetime urban nonsmokers.

Expanding the pollution contrast

We have examined the effects of greater air pollution contrasts than existed between Ansonia and Lebanon in three ways: (1) by considering health effects of high indoor pollutant levels in homes, (2) by comparing our findings with those of others, in areas with pollutant concentrations higher than those in Ansonia, (3) by examining respiratory health data from people living in clean rural areas.

Fig. 1 Per cent prevalence of usual phlegm by sex, age, smoking and residence (LR, PU and LU explained in Table 2). Predicted prevalences are those obtained from the model summarised in Table 3. Only significant variables are shown; for example, smokers were not subdivided according to residence as this variable was only significant among nonsmokers.



(1) Suspended particulates indoors are predominantly small-sized ($<1\ \mu m$ diameter) and thus able to penetrate into small bronchi and alveoli[19]. Hence, with equal TSP concentrations, indoor air might be more damaging to health than outdoor air, in which large particles are kept suspended by air currents. However, we found no evidence that the high TSP levels (up to $400\ \mu g\ m^{-3}$) in homes with smokers (ref. 20 and H. R. Hosein *et al.*, unpublished observations) were associated with increased symptoms or lung function loss among nonsmokers in the same homes. Nor did we find evidence for loss of lung function among children living in homes with parents who smoked[21]. Thus, much higher TSP concentrations than those which occurred outdoors in Ansonia may still be subthreshold with respect to health effects.

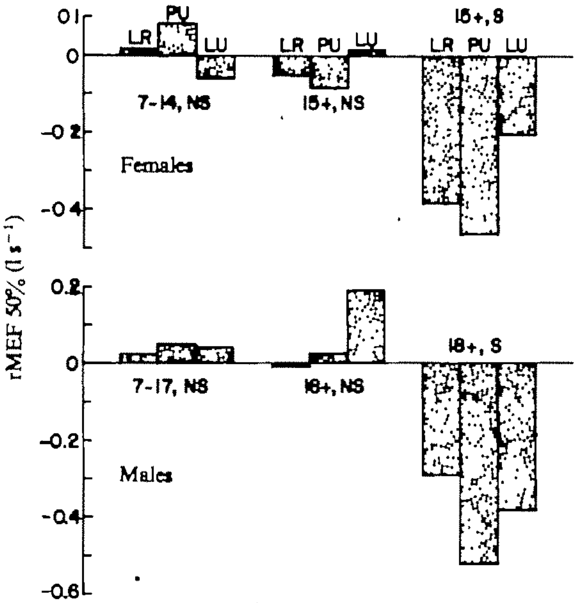


Fig. 2 Mean residual MEF50% (instantaneous maximum flow at 50% FVC). Residual (rMEF50%) = observed − predicted value. None of the differences between residential groups (LR, PU, LU, see Table 2) are significant in any of the six groups by sex, age and smoking habits. For boys and girls, this was also true when a further subdivision was made according to age (7–9 yr, 10–14 yr, 15–17 yr), and differences between LR, PU and LU residents were examined within each of these age and sex groupings. Differences between adult smokers and nonsmokers were significant within each residential subgroup (for example, LR smoking compared with nonsmoking males or females). For purposes of illustration, MEF50% is shown in $l\ s^{-1}$. The statistical analyses were, however, done on residuals in transformed units, ln MEF50% in children and $\sqrt{\mathrm{MEF50\%}}$ in adults; these transformations were required to obtain satisfactory prediction equations (see ref. 12 for the equations and their derivation). The equations with transformed units provide residuals which are normally distributed and have equal variances independent of age, height and weight within each subgroup by sex and age group. Values for s.e.m. residuals range over 0.037–0.085 in females and boys, and 0.1113–0.1755 in adult male groups. Differences between mean residuals in smoking groups may reflect differences in amounts smoked; although differences in amounts smoked were not significant between residential groups, PU men and women smoked more than LR and LU men and women.

(2) SO_2 and TSP concentrations have previously been high in many urban areas. In the UK, very high pollution levels (up to $1{,}160\ \mu g\ m^{-3}\ SO_2$) which occurred in 1965 and previous years, were clearly associated with exacerbations of bronchitis[22]. However, pollution levels lower than those in the UK in the 1950s and early 1960s do not seem to have any marked effect on health. For example, results similar to ours (a slight excess of cough and phlegm) were obtained among men employed in Manhattan in 1962–63 (ref. 23), before current air pollution control programmes began, and at a time when SO_2 and TSP were of the order of $500\ \mu g\ m^{-3}$ and $250\ \mu g\ m^{-3}$, respectively[13]. At about the same time (1961–62), nonsmoking black and white male postal or transit workers in New York City[24,25] had $FEV_{1.0}$ values almost identical to those in our urban and rural nonsmokers in 1973 when age, race and height are taken into account. According to our equations describing growth of lung function in children[12], Czech children (age 10–11 yr) living in a town with high SO_2 (annual mean $150–170\ \mu g\ m^{-3}$)[26] and TSP (annual mean $100–110\ \mu g\ m^{-3}$)[26] concentrations have lung function values[27] very close to those of 10–11-yr-old children in Ansonia and Lebanon.

Oxidant pollution has not been clearly linked with increased prevalence of chronic bronchitis or its component symptoms; in fact, chronic bronchitis seems to be about as uncommon among Los Angeles nonsmokers[28] as among our rural (LR+PU) nonsmokers of the same age (45–64 yr), that is, 1.7 and 1.5%, respectively. Nonsmokers in Los Angeles, exposed to higher oxidant and TSP concentrations, and nonsmokers in Connecticut have similar lung function when height is taken into account (Table 4).

(3) The lack of important contrasts in air pollution and respiratory health between Ansonia and Lebanon might be explained if Lebanon were a relatively polluted rural area. However, we have found no evidence that respiratory health is demonstrably better in pristine rural areas. Nonsmokers in such an area (Chilliwack, British Columbia) had higher prevalences of cough and phlegm[29] than our lifetime urban nonsmokers. Residents of Winnsboro, South Carolina (with significantly lower SO_2 and NO_2 concentrations than those of Lebanon[1]) had symptom prevalences and lung function similar to those of Lebanon residents, and a higher prevalence of a history of asthma (unpublished observations). Black children and adults in a primitive village in Upper Volta, away from cities and with minimal automotive traffic[30], had FVC and $FEV_{1.0}$ values similar to those we recorded among urban black residents in Ansonia and rural black residents in South Carolina.

We conclude from these comparisons between our data and those of others that even relatively low levels of air pollution (as in Ansonia) may be associated with a slight excess of some respiratory symptoms in nonsmokers, but that these symptoms may only become noticeably worse when SO_2 and particulates reach levels higher than those prevailing in Manhattan in 1962–63. Even high concentrations of SO_2, TSP and oxidants by current US standards are not associated with loss of lung function when sex, race, age, height and weight are adequately taken into account.

Implications

The overall impact of outdoor air pollution on respiratory health in Ansonia residents seems to be minimal. Even though the levels of air pollution in Ansonia are low by the standards of the UK in the 1950s, this is of interest because Connecticut is frequently said to have "the worst air pollution in the country outside Los Angeles" (ref. 31). However, any effect of air pollution in Ansonia is small compared with the effects of cigarette smoking and of certain occupational exposure hazards, such as occur among cotton textile workers[5]. Nor have we found evidence that outdoor air pollution worse than that in Ansonia (for example, in Los Angeles[28], New York[23,24,25], Rotterdam[18] or Czechoslovakia[26,27]) produces substantially greater effects on health. Our conclusions are valid for those lung disorders which affect vital capacity and air flow rates; these include asthma, bronchitis and emphysema. There may be sensitive subgroups within the general population which we have not been able to detect, but this remains speculation.

The extent to which communities want to abate air pollution, and the economical burdens which they are willing to accept for that purpose, are matters for society to decide. There are many reasons, aesthetic as well as hygiene-related, for reducing urban air pollution. Within rather wide margins, however, variations in air quality do not seem to have substantial effects on the prevalence and severity of common diseases which affect airway calibre.

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Molecular structure of a double helical DNA fragment intercalator complex between deoxy CpG and a terpyridine platinum compound

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The crystal structure of a complex containing deoxy CpG and a terpyridine platinum compound (TPH) shows a DNA double helical fragment with TPH intercalated between two Watson–Crick GC base pairs. The DNA unwinding angle is 23° and the pucker of the deoxyribose rings differ at the 3' and 5' ends.

STUDY of the molecular structure of nucleic acid components is of considerable interest in developing an understanding of the macromolecular nucleic acids. Single crystal X-ray diffraction analysis allows us to determine unambiguously several geometrical details concerning these molecules. Studies from this laboratory[1–4] showed that it was possible to crystallise self-complementary ribodinucleoside phosphates as double helical fragments with Watson–Crick hydrogen bonding between the bases. An interesting application of these studies is their extension to double helical fragments which crystallise with planar molecules intercalated between their base pairs. Lerman[5] postulated that certain classes of planar molecules, many of which are mutagenic or carcinogenic, act by insertion between adjacent base pairs in the DNA double helix. Several structural studies have supported this interpretation. Sobell and his colleagues[6,7] described the structure of an RNA double helical fragment which contained an intercalator and several different structures of this type have now been solved with a variety of intercalators lodged inside double helical ribonucleotide fragments[8–11]. These crystallographic studies show that the pucker of the ribose ring is modified by the insertion of an intercalator between the base pairs. However, a major difference between DNA and RNA double helices is the different pucker of the sugar ring. It is therefore of interest to ask how intercalation will modify the geometry of the DNA backbone especially with regard to the pucker of the deoxyribose ring[12].

In this article we report the crystal structure of a complex containing deoxycytidylyl-(3',5')-deoxyguanosine (deoxy CpG) which has crystallised with the intercalator 2-hydroxyethanethiolato-2,2',2''-terpyridine-platinum (II) [TPH][13,14]. In this structure, deoxy CpG forms an antiparallel double helix in which the helix has unwound and the base pairs are unstacked with one TPH molecule intercalated between the base pairs of the double helical fragment. Another TPH molecule is stacked between double helical fragments in the lattice. This structure allows us to determine directly both the unwinding angle of the DNA double helical fragment as well as the pucker of the deoxyribose rings.

Experimental methods

The dimer deoxy CpG was prepared using the phosphotriester method[15,16]. The dimer thus obtained was converted into the ammonium salt by passing it twice over a Dowex cation-exchange resin in the NH_4^+ form. The material was freeze dried

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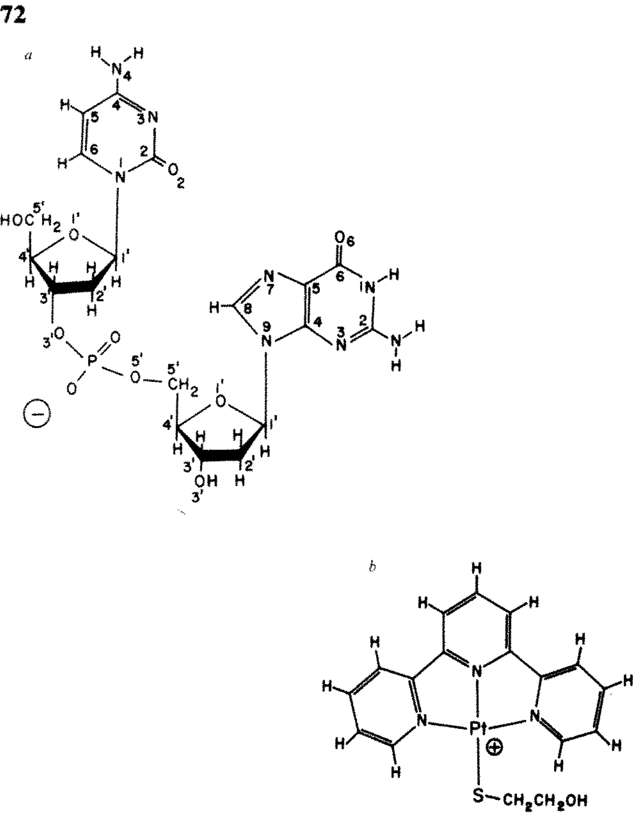

Fig. 1 Structural formulae of *a*, deoxycytidylyl-(3′,5′)-deoxyguanosine and *b*, TPH.

to a fluffy solid. The product was homogeneous and contained 3′–5′ internucleotide linkages only (as shown using high pressure liquid chromatographic analysis and enzymatic digestion by spleen and venom phosphodiesterases). The intercalator TPH was prepared as described by Lippard and his colleagues[13,14].

Crystals were grown using the vapour diffusion method at room temperature in a manner similar to that used in crystallising tRNA[17]. An aliquot of 80 μl of an aqueous solution containing 3mM NH_4^+ deoxy CpG, 3mM of the intercalator TPH nitrate, 25mM sodium cacodylate buffer (*p*H 6.0), and 17.5% 2-methyl-2,4-pentanediol was placed into a depression of a spot plate. The plate was sealed in a small chamber over a reservoir solution containing 75% 2-methyl-2,4-pentanediol. Orange-red plate crystals were obtained within 1 week. The UV spectra of dissolved crystals indicated a one-to-one stoichiometry of both the intercalator and the nucleotide. The material crystallised in the space group $P2_12_12_1$ with $a = 22.25$ Å, $b = 13.57$ Å, and $c = 33.12$ Å. Examination of precession photographs showed very strong reflections at 040 and 020 which correspond to planes at intervals of 3.4 and 6.8 Å along the *b* axis. This suggested stacking of base and intercalator planes perpendicular to the *b* axis which was verified by the structure determination. Data were collected at 8 °C with Ni-filtered CuK_α X-ray radiation on a Picker FACS-I diffractometer to a resolution of 1.1 Å. 3,519 independent reflections were measured with intensities greater than two standard deviations over their background. Data were corrected for Lorentz and polarisation factors as well as for absorption. A Patterson map revealed the position of two platinum peaks—one sharp and one moderately disordered. The structure was determined by difference Fouriers ($F_o - F_c$) and sum function Fouriers ($2F_o - F_c$) and was then refined by the least-squares method in successive cycles using a PDP 11-50 computer. The Nonius structure determination package was used. At the present stage of the refinement, the residual factor (R-factor) has been reduced to 15%. In addition to locating the intercalators and nucleotides, 20 water molecules have been identified in the asymmetric unit.

Results

Figure 1 shows the structural formulae of deoxy CpG and the intercalator TPH—singly charged molecules of opposite sign. The 2:2 complex is thus electrically neutral and no other ions are found in the lattice. Figure 2 shows a view of the molecule looking down the *a* axis in which the planar intercalator and base pairs are visualised virtually end on. In this view, it can be seen that the guanine and cytosine bases are paired together with three hydrogen bonds as is generally assumed to occur in DNA. The TPH in the centre of the diagram is intercalated between the two base pairs in the double helical fragment. The deoxyguanosine at the 3′ end of the molecule has a deoxyribose with the C2′ *endo* pucker, which is the normal pucker found in double helical B DNA[18]. However, the deoxycytidine at the 5′ end has a modified pucker, namely C3′ *endo* which is usually found in double helical RNA[19] but is not found in normal B DNA. At the top and the bottom of the diagram are two intercalator molecules which are located at either end of the small double helical DNA fragment. These are related by a unit cell translation along the *b* axis, and are moderately disordered in the plane of the molecule. They are found principally in two locations, approximately 3.2 Å apart, located on either side of the pseudo-2-fold axis which is found parallel to the *a* axis at this level. The two sites occupied by this TPH molecule have nearly equal occupancies. Because of this disorder, the hydroxy-ethyl side chains of these stacking TPH molecules are not shown in Fig. 2. The complex has a non-crystallographic pseudo 2-fold axis passing through the intercalated TPH molecule parallel to the *a* axis. The hydroxy-ethyl side chain which is attached to the central intercalating TPH molecule effectively prevents the existence of an exact 2-fold axis at that point since the side chain does not have 2-fold symmetry as seen in Fig. 2.

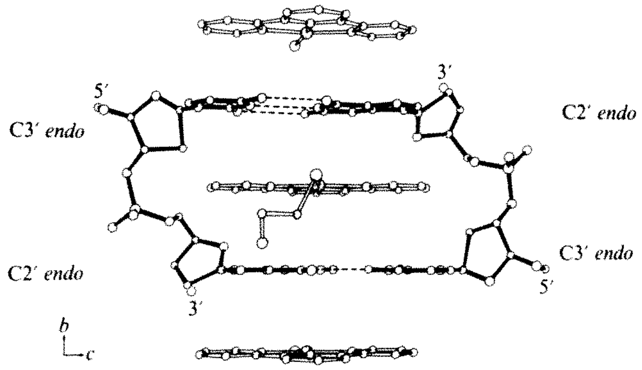

Fig. 2 View of the structure as seen down the *a* axis. The hydroxyethane side chains are not shown for the intercalators at either end of the double helical fragment. The glycosidic torsion angle (χ) is seen to be different at the two ends of the deoxy CpG. The two deoxyguanosine torsion angles are 114° and 117°; the angles for deoxycytidine are 32° and 34°. The phosphodiester linkage has a *gauche-gauche* conformation. Oxygen, sulphur, phosphorus and platinum atoms are drawn with larger circles than carbon atoms; the platinum atoms are stippled. Hydrogen bonds are drawn as dashed lines.

Figure 3 is a diagram of the double helical DNA fragment containing the intercalating TPH molecule. This is a view perpendicular to the base pairs and the central intercalator. The difference in the pucker of the deoxyribose rings at the 5′ and 3′ ends of the molecule can be seen. It also shows the extensive overlapping of the π electron systems of the purine and pyrimidine rings with the terpyridine of the intercalator. Interestingly, all of the double helical ribodinucleoside phosphate intercalator structures which have been crystallised also contain the intercalator inserted in a pyrimidine–purine sequence[6-11]. In

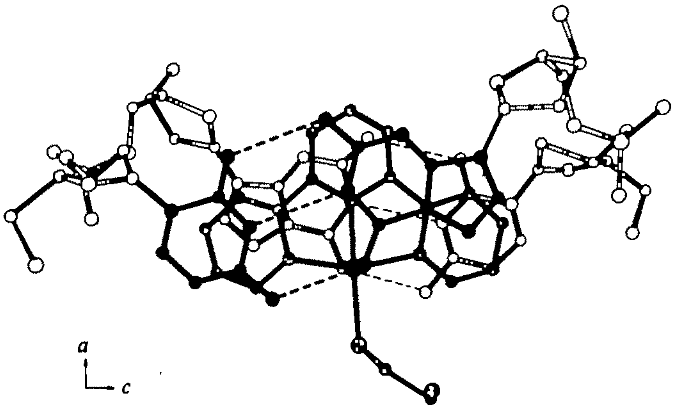

Fig. 3 A view of the structure down the *b* axis. Only the central intercalated TPH molecule is shown with the double helical fragment. The base pair closest to the reader is shaded solid black, the central intercalator is stippled while the bottom base pair is unshaded. The difference in deoxyribose pucker at the two ends of the chain is shown clearly here. Considerable overlap is evident between the bases and the intercalator pyridine rings. A pseudo-2-fold axis can be seen along the sulphur–platinum bond.

the native double helix there is less base stacking at the sequence pyrimidine–purine than at the sequence purine–pyrimidine. The stacking in the present pyrimidine–purine structure is considerably enhanced by the insertion of the intercalator, as seen in Fig. 3. For example, the outer pyridine rings are in a position where they provide a considerable degree of overlap for the purine ring on one side and the pyrimidine on the other. In a similar way the central pyridine ring of the intercalator overlaps the two purine residues in the centre of the molecule. Note that the oxygen O6 of both guanine molecules are located virtually above and below the central platinum atom in the intercalator, 3.4 Å away. It remains to be seen whether this feature plays a part in the interaction between this intercalator and the base pairs. It should be noted that the three unsaturated pyridine rings are organised in an arc which effectively overlaps the base pairs above and below. A similar arc-like arrangement is found in several of the unsaturated condensed ring intercalators such as ethidium and ellipticine$^{6-9}$. As is shown in Figs 2 and 3, the side chain of the intercalating molecule is located in the major groove of the double helix. This orientation is in contrast to the TPH molecules which are stacked at either end of the double helical fragment. Here the side chain is located on the opposite side close to the narrow groove of the double helical fragment.

It can be seen in Fig. 3 that the TPH is intercalating in a symmetrical manner in this complex which is quite different from that which is seen in the ribonucleotide intercalation structures containing acridine orange, 9-aminoacridine and ellipticine^{9,11}.

The intercalator–double helical fragments are stabilised in the crystal lattice by a number of intermolecular interactions. These include hydrogen bonds from the hydroxyl group of the TPH intercalator to the cytosine O2 of another complex. There are also hydrogen bonds between the C5′ OH of deoxycytidine to phosphate oxygens of an adjoining molecule which is related to it by twofold screw axes along the *c* axis. In addition, O3′ of the deoxyguanosine residue is hydrogen bonded to O2 of the cytosine in an adjoining molecule. There are a number of water molecules which form both intramolecular and intermolecular hydrogen bonding bridges. An interesting intramolecular bridging hydrogen bonded water molecule is found connecting the C5′ hydroxyl group and the phosphate oxygen of the same polynucleotide chain. This is responsible for the conformation of the C5′ terminus of the oligonucleotide. However, this bridging water molecule is not likely to be present when the DNA is present in a normal double helical conformation.

It can be seen in Fig. 3 that the two GC Watson–Crick base pairs are related to each other by a right-handed helical rotation. In the B form of DNA the vector between the C1′ atoms of the deoxyribose residues of the hydrogen bonded base pair are related to that of the next base pair by a rotation of 36°, as there are 10 base pairs per turn of the helix. In the present structure, however, the base pairs are no longer 3.4 Å apart but rather 6.8 Å apart due to the intercalator separating them. The inter-strand C1′–C1′ vector is related to the corresponding vector on the other side of the intercalator by an angle of 13°. Thus there is an unwinding of the double helix of 23° relative to that which it would normally have in the absence of the intercalator.

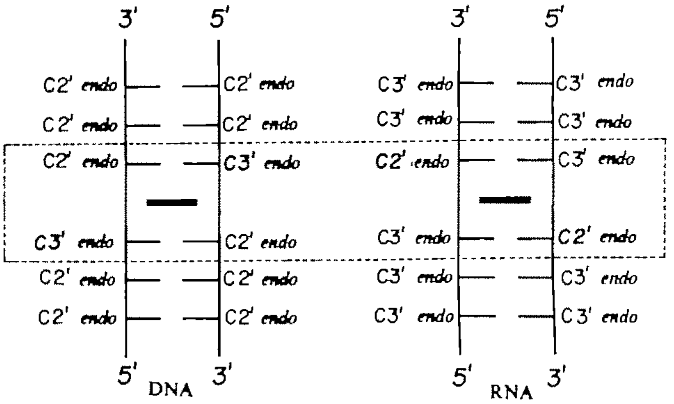

Fig. 4 A schematic diagram showing the sugar conformation in double helical DNA and RNA. The DNA is in the normal B form. The conformational effects of an intercalator are illustrated in the dashed region where the changes in sugar pucker are shown in bold face. For simple intercalators, the conformation in the dashed region is the same for the DNA and the RNA double helix.

Discussion

The intercalator used in this study has several experimental advantages. The presence of the unsaturated terpyridine rings provide a substantial degree of overlap with the adjacent purines and pyrimidines. In addition, the centrally coordinated platinum atom significantly facilitates solution of the X-ray diffraction problem. This intercalator has been used extensively by Lippard and his colleagues in a variety of studies as reviewed recently^{20}. Intercalators of this type compete with ethidium and induce viscometric and spectroscopic changes which can be readily interpreted as due to interaction with DNA in an intercalative mode.

This crystal structure shows the manner in which the DNA double helical fragment accomodates the interposition of a planar molecule between the base pairs. In view of the extensive previous work with intercalators in fragments of an RNA double helix (Table 1), we can make a comparative study. The important difference between the DNA and the RNA double helix is the presence of the additional 2′ OH group in the ribose of RNA, which makes RNA adopt a C3′ *endo* ring pucker while in DNA the deoxyribose is normally C2′ *endo*^{18,19}. This nomenclature refers to the displacement of either the C2′ or C3′ carbon atom out of the plane of the ring in a direction towards the base or the major groove of the double helix. One of the features of several RNA double helix–intercalator structures (Table 1) has been the observation that puckering of the ribose rings differs at the 3′ and 5′ ends of the intercalated segment. This is especially true with 'simple' intercalators which do not form hydrogen bonds with the nucleotide backbone. The same phenomenon is seen in the present structure. The DNA fragment shows a C3′

Table 1 Intercalation geometry of nucleic acid structures*

	Dinucleoside phosphate	Intercalator	Sugar pucker 5′ end	Sugar pucker 3′ end	Twist angle	Ref.
DNA fragments	dCpG	TPH$^+$	C3′ *endo*	C2′ *endo*	13°	This work
RNA fragments	rIodoUpA	Ethidium$^+$	C3′ *endo*	C2′ *endo*	8°	6, 7
	rIodoCpG	Ethidium$^+$	C3′ *endo*	C2′ *endo*	8°	8
	rIodoCpG	9-NH$_2$-Acridine$^+$	C3′ *endo*	C2′ *endo*	8°	9
	rIodoCpG	Ellipticine$^+$	C3′ *endo*	C2′ *endo*	10°	11
	rIodoCpG	Acridine Orange$^+$	C3′ *endo*	C2′ *endo*	10°	11
	rIodoCpG	Proflavine$^+$	C3′ *endo*	C3′ *endo*	36°	11
	rCpG	Proflavine$^+$	C3′ *endo*	C3′ *endo*	33°	10

* Two different classes of intercalators are indicated by the dashed line.

endo conformation at the 5′ end of the dinucleoside phosphate and the normal C2′ *endo* conformation at the 3′ end, that is C3′ *endo* (3′, 5′) C2′ *endo* mixed sugar puckering. In the simple intercalated RNA helical fragments it is the 3′ end of the ribonucleotide chain which is perturbed to adopt the C2′ *endo* conformation which is normally found in the B form of DNA but not normally found in the double helical RNA chain. There are two interesting exceptions which occur with proflavine intercalation as shown in Table 1. These differences are likely to be associated with the fact that the amino groups on proflavine form hydrogen bonds with the phosphate group of the internucleotide link. This may have the effect of constraining the pucker which the ribose residues can adopt.

For the class of 'simple' intercalators which intercalate but do not hydrogen bond with the phosphate groups we see an interesting similarity in the manner in which the DNA double helix and the RNA double helix accomodate the intercalation (Fig. 4). Away from the intercalated site, the sugars of the two types of double helices have the different puckers mentioned above. At the intercalator site (Fig. 4) both polynucleotide chains have a similar C3′ *endo* (3′,5′) C2′ *endo* conformation. This result supports the use of intercalated RNA helical fragments as models for studying the geometry of DNA intercalation. Such a mixed puckering conformation has also been suggested to occur in B DNA by a model building study[21]. This identity of conformation in the region surrounding the intercalators is due to a change in the conformation of the DNA segment at the 5′ end of the internucleotide linkage surrounding the intercalator moving towards the conformation found in the RNA double helix. In the RNA double helix, a new conformation is adopted at the 3′ end, the C2′ *endo*, which is normally associated with the DNA double helix. The net effect of both of these changes is that it gives rise to the familiar nearest-neighbour exclusion effect which is found with intercalation. When double helical DNA or RNA is fully saturated with intercalators, only every other site can be occupied. This is because the conformational change adopted by the polynucleotide chain in accomodating the intercalator differs at the 3′ and 5′ end. One cannot obtain this conformation between every base pair but at best between every other base pair. An X-ray diffraction study of DNA fibres using the same terpyridine platinum complex used in this study demonstrated the nearest-neighbour exclusion in DNA fibres[22]. The interpretation was facilitated by the presence of platinum in the intercalator which scatters X rays strongly.

One of the features associated with intercalation in a DNA double helix is the fact that it gradually unwinds the helix. Studies of ethidium intercalation into supercoiled DNA has made possible a calculation of the unwinding angle in DNA associated with each molecule of ethidium[23]. The studies indicate that insertion of ethidium between the base pair decreases the twist angle between the base pairs by 26° ± 2°. This value is very close to the 23° observed in the present structure and is also similar to the results seen with the insertion of simple intercalators into ribonucleotide fragments. In contrast, the intercalator proflavine which hydrogen bonds to the phosphate groups has an unwinding angle of 0°.

In the present crystal structure one of the TPH molecules is used to maintain continuity of stacking between one double helical fragment and the next. That molecule is disordered in an interesting manner. In one of the two disordered positions, there is a maximum degree of overlap with the guanine residue in the double helical fragment below it. In the other disordered position, there is a greater degree of overlap with a guanine residue from the double helical fragment above it. One element in the disordering process may be due to the presence of two positions with similar energy minima. There is no disorder in the intercalated TPH molecule.

Single crystal analyses of nucleic acid fragments have considerable value in providing us with fine details associated with the manner in which these molecules adapt to a variety of environments. The present investigation has illustrated the manner in which the DNA backbone accomodates the insertion of an intercalator between base pairs. Studies similar to these, and especially those involving longer deoxyoligonucleotides should prove of great value in enhancing our understanding of the mode of action of DNA in biological systems.

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letters

Pulsed and unpulsed light from the Vela and Crab pulsars

OBSERVATIONS of the Vela and Crab pulsars were made with the Image Photon Counting System[1] (IPCS) at the *f*/15 focus of the 3.9 m Anglo–Australian Telescope (AAT), on 9 and 10 March 1978. Observations of the unpulsed light from the Crab pulsar have been obtained with an electronographic camera[5] used with the 0.9 m Yapp reflector of the Royal Greenwich Observatory (RGO). We obtained phase-resolved pictures of the Vela pulsar[2,3], positively identifying it with the faint, blue candidate star[4] and detecting a relatively strong, unpulsed light component. We also detected a weak unpulsed component from the Crab pulsar.

In the AAT observations the pulsar period was divided into eight phase intervals, and a picture was accumulated of the area around the pulsar during each phase interval. The eight pictures, each of 42×42 pixels (corresponding to 12 arc s on a side), were held in the memory of an Interdata-70 computer. Every millisecond, an interrupt from the observatory timing system caused the computer to calculate the current phase of the pulsar, and select to which of the eight pictures the next TV frame from the IPCS would be delivered. In this way, TV frames from the IPCS, generated every 6.2 ms, were stored and each picture in the computer memory became the integration of many frames from the same phase interval. Total integration times on the Vela pulsar were respectively 5 h 16 min (B) and 3 h 30 min (U). Crab pulsar observations were for ~10 min each with the telescope stopped down to 1.8 m aperture (central obstruction of secondary 1.4 m); this was to prevent saturation of the IPCS during the bright portion of the pulse. The Vela pulsar observations were all phased with respect to the dedispersed radio pulse arrival time, 21 ms before the first optical pulse. Predictions of radio pulse phase were made from radio measurements made as part of a continuing series of pulsar timing observations[6]. The Crab pulsar observations were obtained at arbitrary phases.

During the observations, the eight phase-resolved pictures were cyclically displayed on a TV monitor, so that the pulsar pulses were seen in stroboscopic fashion. Photographs of the display are shown in Fig. 1 for observations in blue light (B-band) for the Vela and Crab pulsars. Figure 1 confirms the identification[3] of the Vela pulsar with the star known[4] as M and clearly shows residual emission in its light curve outside the two main peaks, which fall in phases 2 and 4.

The intensities of stars in each field observed with the IPCS were determined by a least squares procedure which simultaneously fitted the sky background (three parameters), the positions and intensities of the stars (three parameters per star), and the stars' profile expressed as a two-dimensional semi-empirical point-spread function with central core and extended wings (five parameters). Nebulosity in both the Crab and Vela pulsar fields could produce systematic errors in the measurements of the pulsar intensities, and so was determined from the data by an iterative technique. The residuals resulting from an initial fit were smoothed by a broad spatial filter to determine the background variations whose scale was large, compared to the size of a star, but which was small compared to the size of the field. This picture, representing the background nebulosity, was subtracted from the picture of the pulsar field, and the star intensities were obtained by reapplying our fitting procedure. The intensities of the Crab and Vela pulsars in the B and U filter bands are given as a function of pulse phase in Fig. 2.

A surprising result from the observations is the intensity of the emission from the Vela pulsar outside the two main peaks. The unpulsed emission accounts for about half the total emission from the pulsar. To estimate the intensity of the unpulsed light we have fitted higher resolution light curves to the data in the following way.

Each of the pictures within a set of eight covers an interval of pulsar phase. The edges of this interval are blurred by the IPCS response time and by the millisecond timing interrupts. Thus the pulsar intensities derived from those pictures which give the lowest intensities may include pulsed light from adjacent phase intervals in which the pulsars were brighter. This problem is severe in the case of the Crab (period 33 mins, phase interval 4 ms) but not as severe for Vela (period 89 ms, phase interval 11 ms). Light curves obtained in 1977 with an unfiltered S20 photomultiplier and high time resolution[3] have been convolved with the appropriate smoothing function representing the instrumental smearing. Assuming that the shape of the light curve is unchanged in the past year and that the shape of the pulsed component does not depend much on the colour in which it is observed, we fitted the amplitude and baseline of the convolved light curve by a least squares procedure to the pulsar intensity derived from the IPCS observations. The fit of the convolved light curves to the observations is shown in Fig. 2*a*. The high time resolution light curves used for the convolution are plotted in Fig. 2*b*; for each curve the true zero is represented by the base of the plot.

The apparent magnitudes in U and B of the time averaged

Table 1 Pulsar magnitudes

	Crab	Vela
Mean apparent magnitude (B)	—	23.56 ± 0.03
Mean pulsed apparent magnitude (B)	17.0*	24.4
Mean steady apparent magnitude (B)	18.6	24.3
Mean apparent magnitude (U)	—	23.20 ± 0.14
Mean pulsed apparent magnitude (U)	16.5*	23.8
Mean steady apparent magnitude (U)	18.1	24.2
Distance[11] (pc)	2,000	500
Distance modulus (m–M)	11.5	8.5
Absorption[3,12] (A_B)	2.1	0.5
Absorption[3,12] (A_U)	2 8	0.7
Mean steady absolute magnitude (M_B)	5.0	15.3
Mean steady absolute magnitude (M_U)	3.8	15.0
Ratio of steady/pulsed light expressed as a difference in magnitude (B)	1.6	−0.1
Ratio of steady/pulsed light expressed as a difference in magnitude (U)	1.5	0.4

* Ref. 10.

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intensity from the Vela pulsar are given in Table 1. Calibration of the blue sensitivity of the IPCS was with respect to faint standard stars in the globular cluster ω Centauri (R. Cannon, personal communication), and the standard error quoted for the blue magnitude represents the internal consistency of the data, as the calibration is relatively well assured. Calibration of the UV sensitivity of the IPCS was with respect to star K (ref. 3) and with respect to UV measurements[7] of brighter stars in ω Cen (no faint U standards are available). The brighter stars were observed with the telescope out of focus in an attempt to avoid saturation problems of the IPCS. Because this calibration is less assured, a larger standard error for the U measurement is given based on the agreement within the methods.

Table 1 also gives estimates of the magnitudes of the pulsed and unpulsed light from each pulsar. The model chosen to distinguish pulsed and unpulsed light is that the high-resolution light curves representing the pulsed emission are supported on a steady pedestal of constant emission.

The presence of the small unpulsed background from the Crab pulsar, which amounts to 3.6 ± 0.7% of the peak intensity

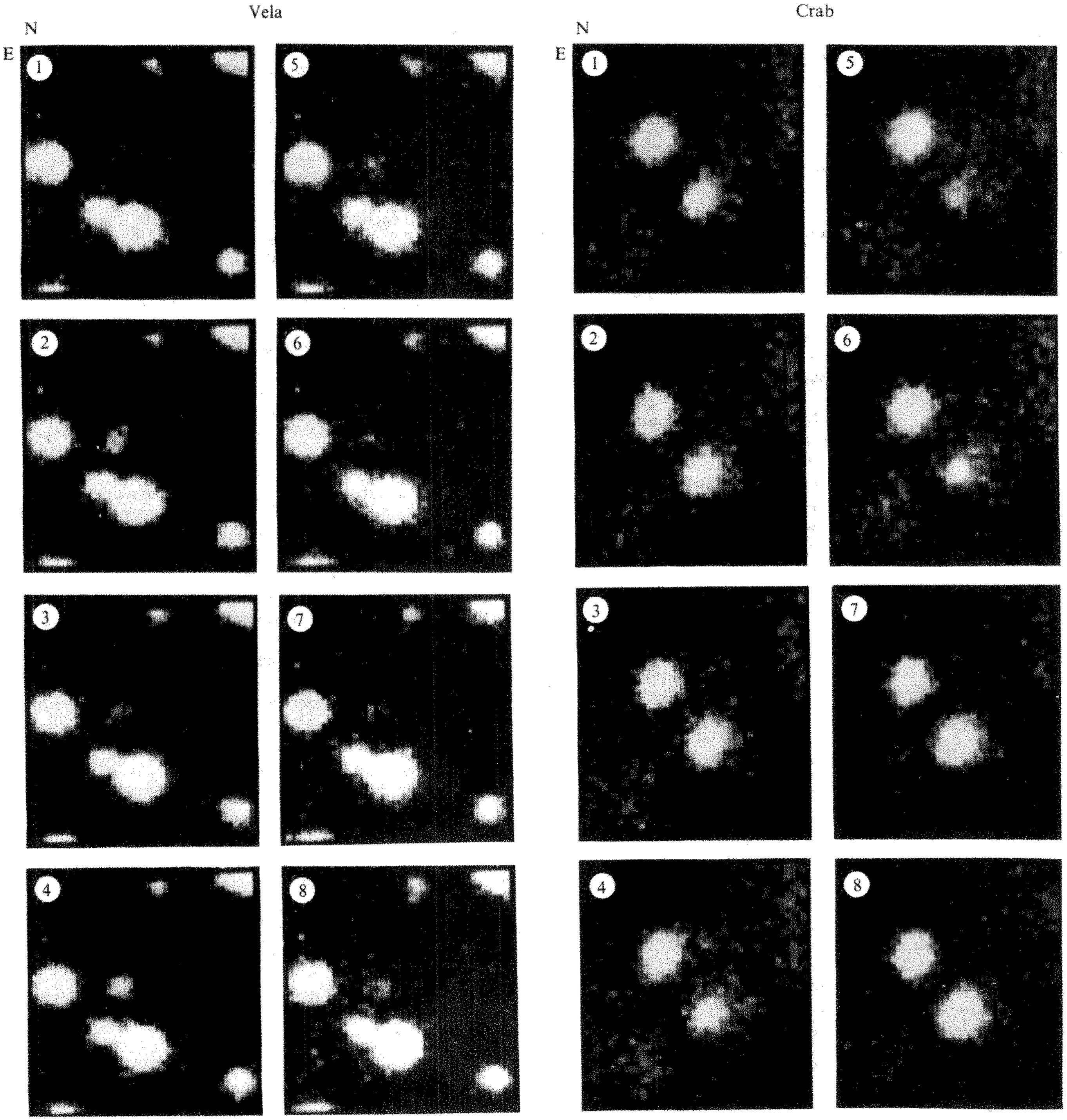

Fig. 1 The fields of the Crab and Vela pulsars in blue light as shown by the observing display at the Anglo-Australian Telescope. Each field covers an area on the sky 12 × 12 arc s, and one eighth of the phase of the pulsar's cycle. The fields are numbered in sequential phase order for each pulsar. The main pulse of the Crab pulsar occurs in phases 7 and 8, while the inter-pulse occurs in phases 2 and 3. The main double pulse of the Vela pulsar occurs in phases 2 and 4. Emission can be seen throughout the whole cycle of the Vela pulsar. (Emission seen throughout the whole cycle of the Crab predominantly results from instrumental smearing.)

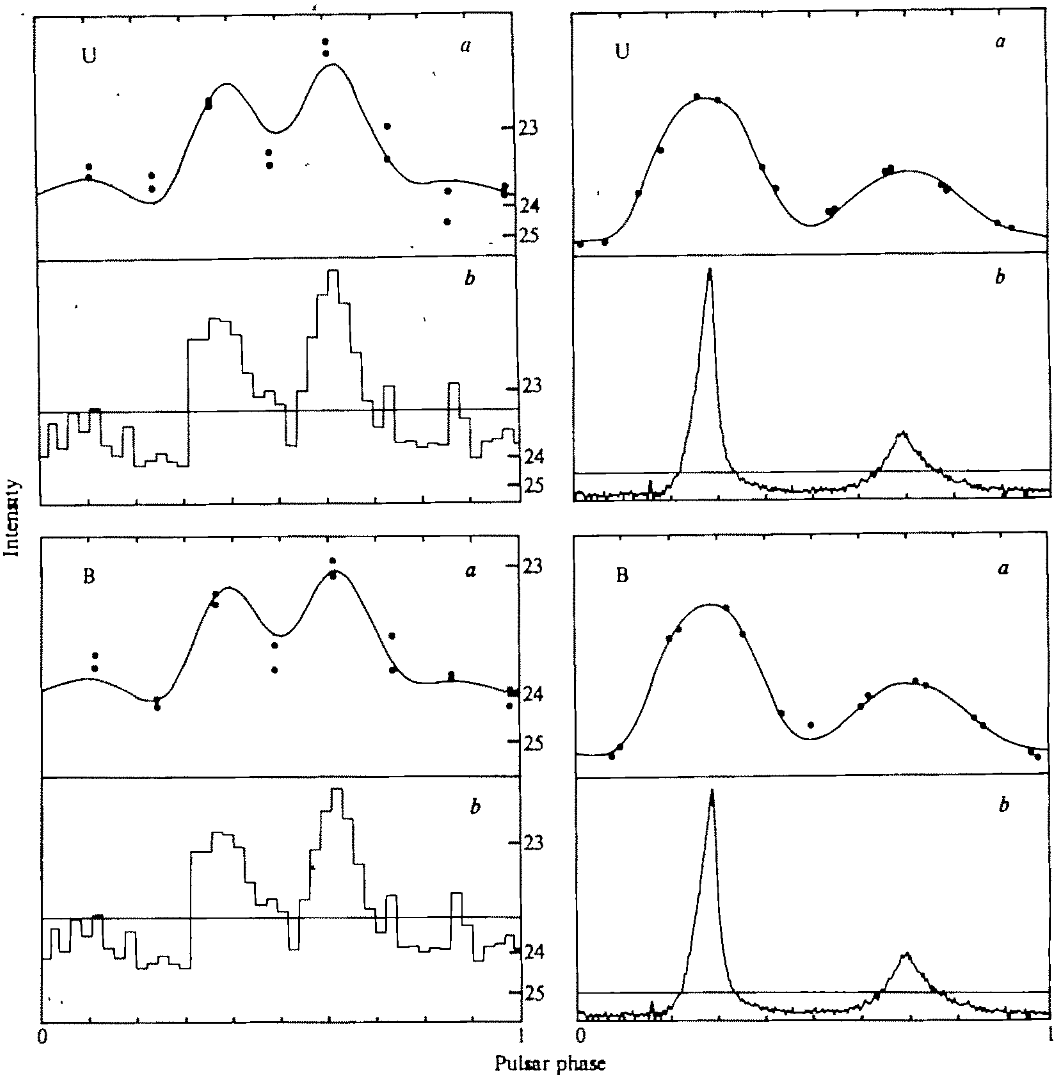

Fig. 2 Light curves of the Crab and Vela pulsars in UV (U) and blue (B) light. Points represent the intensity of the pulsars as observed with the IPCS. (The data were divided into halves of equal weight to generate two points per phase interval.) As described in the text, curves were fitted to the data points by smoothing high-time-resolution profiles obtained with unfiltered S20 photomultipliers in earlier observations[2]. The high-time-resolution profiles are shown in the lower panels (*b*) with their mean intensity indicated by the horizontal line. In all eight panels of this figure, the ordinate represents light intensity correctly zeroed at the base of each panel. Calibration to magnitudes is given on the right hand ordinate, for the Vela pulsar. The abscissa of each panel covers one cycle of the pulsars.

(but a suprisingly large 25% of the mean pulsed light) is not in serious disagreement with the estimated upper limit of 2% of maximum intensity given by Miller and Wampler[4] on the basis of estimates from a TV picture taken through a strobing shutter with effective phase resolution of 20% of the pulsar period.

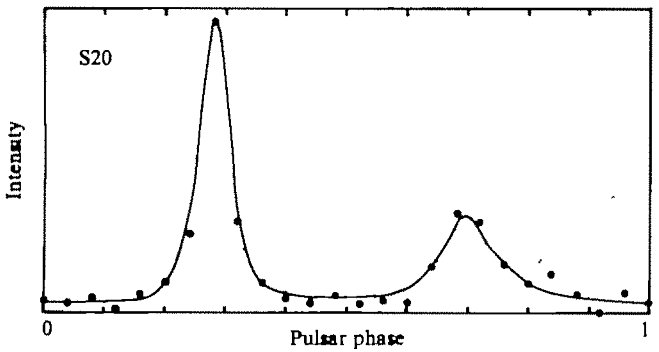

Fig. 3 Points are electronograph measurements of intensity of Crab pulsar summed in 25 equal sized phase bins: curve is high-time-resolution light-curve smoothed to 1.33 ms resolution and adjusted in amplitude, phase and base level to fit data. The minimum point of the curve (preceding the main pulse) is significantly above the zero level

The unpulsed background of the Crab pulsar which we observe with the IPCS is confirmed by an independent observation which was also independently reduced. In this observation (made on 7 November 1977) the photocathode of a McMullan electronographic camera[5] was masked to leave only a small area exposed in the focal plane of the RGO 0.9 m telescope (no filter, S20 photocathode). The picture area was stepped by deflection coils in synchronism with the pulsar period, to give 25 separate pictures, one of each phase bin. The response-time of the electronograph to a step of the deflection coils was very small compared with the 1.33 ms dwell-time for a picture. Using a PDS microdensitometer, each electronograph picture was scanned along the line joining the pulsar and its companion to the north-east, and the resulting profile across the pair of images was fitted with two one-dimensional point-spread functions, with similar form to the two-dimensional point-spread function used in the IPCS analysis. The ratio of the heights of the profiles is a measure of the intensity of the pulsar, because the electronograph produces an image whose density is linearly proportional to image intensity. We show the Crab pulsar light curve derived from an observation of duration 10 min in Fig. 3. The unpulsed background of the Crab pulsar detected with the unfiltered S20 electronograph is 3.7±0.8% of its peak intensity, in excellent agreement with the IPCS observations in U and B.

The ratio of steady/pulsed light for the Crab pulsar is similar in B, U (Table 1) and S20 wavebands. However, for the Vela pulsar the steady component may be weaker in the UV band than in the blue band. We used the χ^2-test to measure how similar the two light curves were (to within a scale factor). The probability of the value of χ^2 which measured the goodness of the match between the two observed curves was 4% ($\chi^2 = 14.9$, 7 d.f.). A better match between the U and B light-curves occurred when an extra steady intensity, equal to 0.26 of the mean U intensity, was added to the U light curve ($\chi^2_{\min} = 10.0$, probability 20%). Thus the steady light component of the Vela pulsar may be redder than the pulsed component.

For both pulsars the absolute magnitude of the steady, unpulsed component (Table 1) is too great to be attributed to thermal emission from the neutron star from which the pulsed radiation is beamed. The thermal radiation from a neutron star 9 km in radius was calculated[9] to have absolute magnitude $M_B = 19.4$ if the surface has a temperature $T = 2 \times 10^6$ K and would be $M_B \sim 25$ at $T = 10^4$ K.

There are several possible origins for the steady, unpulsed light component. (1) It may be pulsed light emitted from points distributed in space. Such light would reach the Earth with various arrival times and could contribute light at all phases. (2) A pseudo-steady component will also be seen if the pulses are so broad that they effectively fill the entire period. The high time resolution[3] light curve of the Vela pulsar, (Fig. 2*b*) hints at two weak pulses, similar to the two main pulses, but 180° offset in phase. The two main pulses may be produced by one pole of the rotating neutron star while the two weaker pulses are produced by the other pole. (3) The steady component may be thermal or other radiation from material near the pulsar (such as, a nebular wisp) that is reflecting or reprocessing energy in the pulses. Alternatively, if the beamed emission originates away from the neutron star, at the speed-of-light cylinder for example, the steady emission may be this radiation reflected from the surface of the neutron star.

The indication in Table 1 that the steady component of the Vela pulsar may be redder than the pulsed component, implies that reprocessing may be occurring, although it remains to be explained why almost as much light is received at Earth as a result of reprocessing as is received at Earth directly from the pulsar beam.

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IUE observations of Large Magellanic Cloud members and detection of the 2,200 Å feature

THE dominant feature in the UV spectra of reddened stars in our Galaxy is the broad absorption feature of interstellar origin, centred near 2,200 Å and having a halfwidth of 350 Å (ref. 1). The wavelength position of this feature and the correlation of its strength with other interstellar extinction parameters, for example, the visual colour excess $E(B-V)$ are remarkably uniform throughout the Galaxy within 2 kpc from the Sun[2]. The 2,200 Å feature is attributed to small graphite spheres with radii between 100 and 200 Å (ref. 3), but a molecular origin cannot be ruled out[4]. It is, therefore, the most important feature to search for in the interstellar extinction laws of other galaxies. The Magellanic Clouds are the nearest extragalactic systems and the Large Magellanic Cloud (LMC), in particular the region near 30 Doradus, contains a significant amount of dust[5]. In the past few years considerable doubt has been expressed about the existence of the 2,200 Å feature in the LMC extinction law as derived from surface brightness measurements of the region around 30 Doradus[6], the results being rather inconclusive due to the difficulties arising from uncertainties in the luminosity function for the region concerned[7]. The interstellar extinction law can be reliably determined only from studies of the individual spectra of reddened and unreddened stars of similar spectral types. We present here the UV observations of a moderately reddened and a slightly reddened early type supergiant in the LMC obtained with the IUE. These observations answer the question of whether or not the agents responsible for the 2,200 Å feature are present in interstellar dust in the LMC.

The observations were obtained with the IUE at the low resolution mode ($\Delta\lambda \sim 6$ Å) over the entire wavelength range 3,200–1,150 Å. The spacecraft, its instrumentation, and the performance of the telescope, spectrograph and the SEC cameras are described elsewhere[8,9]. The reddened star is Sanduleak no. SK-69-108 (ref. 10). This star ($V = 12.10$, $B-V = 0.27$) is classified as B3I (ref. 11) and has a visual colour excess $E(B-V) \sim 0.45$. The comparison star, SK-67-108, is situated in a region free from nebulosity. It is classified as B0I (ref. 11) and its reddening, estimated to be $E(B-V) \sim 0.05$ arises mainly in our Galaxy.

Fig. 1 Observed flux distribution of SK-69-108 (*a*) and SK-67-108 (*b*) The points indicated by + signs are due to a fiducial mark.

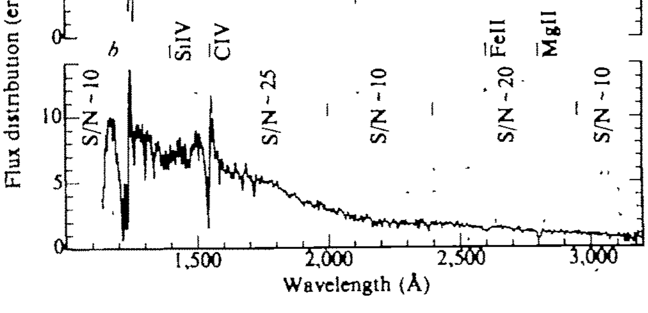

Table 1 Observations

Object	Species type	*U*	*B*	*V*	Image	Aperture	Exposure time (min)
SK-69-108	B3I	11.88	12.37	12.10	LWR1726 LWR1727 SWP1853	Large Large Large	45 90 80
SK-67-108	B0I	11.31	12 36	12.56	LWR1594 SWP1690	Small Small	20 20

LWR = long-wavelength redundant camera; SWP = short-wavelength prime camera.

The reddened star, SK-69-108, was observed with the large entrance slot (15×20 arc s), but the comparison star, SK-67-108 was observed with the small entrance slot (3×3 arc s). The photometric data, exposure times and image numbers are given in Table 1. The reduced spectra were provided by the IUE ESA observatory at Madrid, using the procedures described by Boggess *et al.*[9]. The wavelengths were checked using the positions of the major lines identified in the spectra. The raw image of each exposure was examined for saturated pixels, and only the unsaturated portions of the spectra were used. The net spectrum (observed minus smoothed background) was converted to absolute flux units using the preliminary system sensitivity functions provided by the Appleton laboratory.

Figure 1 shows the spectra of the reddened star SK-69-108 and the comparison star SK-67-108. The two peaks in excess of 20 units near 1,600 and 1,500 Å in the spectrum of SK-69-108 are due to charged particles incident on the camera; this is clear on the raw image. The mean signal-to-noise ratios computed from the counts recorded in the individual pixels of the raw image for different parts of the spectrum of each star are indicated in Fig. 1.

The C IV doublet at 1,550 Å in the spectrum of the unreddened star is very strong and shows a P-Cygni profile; on the other hand, the S IV doublet at 1,400 Å which, in early-type supergiants is usually as strong as C IV lines[12], is rather weak. Similarly, in the reddened star, the C IV doublet is considerably stronger than the S IV doublet. The most prominent line longwards of 2,500 Å is the Mg II doublet at 2,800 Å, and is present in both spectra. The line near 2,600 Å due to Fe II which appears clearly in the spectrum of the unreddened star is obscured in the spectrum of the reddened star by a fiducial mark on the raw image. Even at low resolution the spectra show a large number of lines; their identifications and spectroscopic comparisons with galactic standards will be presented elsewhere.

Table 2 Observed ($m_\lambda - m_{3100}$) colours

(Å)	SK-69-108	SK-67-108
1,300	0.604	−2 571
1,400	0.087	−2.440
1,500	−0.165	−2.381
1,600	−0 296	−2.389
1,700	−0.226	−2.126
1,800	−0.084	−2.000
1,900	0.301	−1.740
2,000	0.732	−1.523
2,100	1 079	−1.243
2,200	0 970	−1.037
2,300	0.726	−0.962
2,400	0.482	−0.934
2,500	0.308	−0.878
2,600	0.163	−0.704
2,700	0.009	−0.648
2,800	0.073	−0.412
2,900	−0.032	−0.368
3,000	−0.067	−0.249
3,100	0.000	0.000

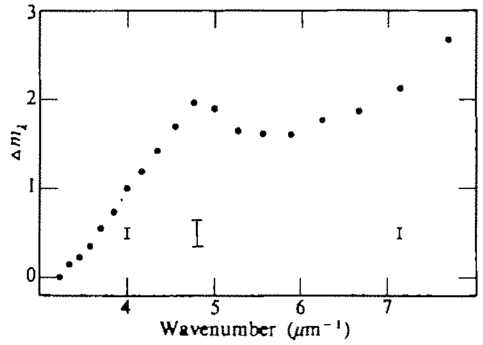

Fig. 2 The extinction law normalised to $\Delta m_\lambda = 0$ at $\lambda^{-1} = 3.23\ \mu\text{m}^{-1}$ and $\Delta m_\lambda = 1$ at $\lambda^{-1} = 4.0\ \mu\text{m}^{-1}$.

To improve the photometric accuracy, photometric bands ($\Delta\lambda = 100$ Å) have been constructed from 3,100 to 1,300 Å at intervals of 100 Å. The colours ($m_\lambda - m_{3100}$) for the reddened and comparison stars are given in Table 2: the errors of these colours range from 0.02 mag for the comparison star to 0.1 mag for the reddened star near 2,200 Å, where the fluxes are very low. The extinction curve Δm_λ against λ^{-1} (μm)$^{-1}$ normalised to $\Delta m_\lambda = 0$ at 3,100 Å and $\Delta m_\lambda = 1$ at 2,500 Å is shown in Fig. 2. These results are preliminary so no correction has been applied for the difference in the spectral types of the two stars.

The determination of the accurate shape of the UV extinction curve in the LMC and the comparison with the galactic extinction law require a larger sample of stars of accurately known spectral types. Although this is beyond the scope of the present work, the observations presented here establish the presence of the 2,200 Å feature in the UV extinction curve in the LMC. The feature which extends from 2,500 to 1,800 Å in the spectrum of SK-69-108 is similar to what is observed in our Galaxy.

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The central source in the supernova remnant G127.1+0.5

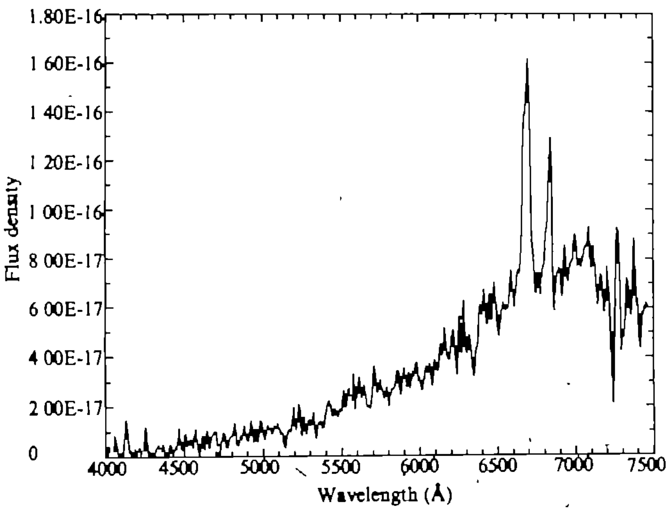
THE discovery[1] of a very small diameter radio source with a very flat spectrum at the centre of the galactic supernova remnant G127.1+0.5 has stimulated much interest[2–4]. Because the central source has unusual properties, and because the *a priori* probability of finding such an object at the very centre of a supernova remnant is exceedingly low, Caswell[1] suggested that the central radio source might be emanating from the collapsed stellar remnant of the supernova that produced the surrounding shell of radio emission. This intriguing idea was pursued by Salter, Pauls and Halsam[3] who showed that the extended supernova remnant is perfectly normal in shape and in radio spectrum, while the central source has a spectrum that is flat from 3 to 15 GHz at a flux density of 0.5 Jansky. A long-baseline interferometric measurement by Shaffer *et al.*[4] at 10.65 GHz showed that the angular size of the central source is about 0.0005 arc s. We now present evidence from direct optical observation suggesting that the link between the central source and the supernova remnant is purely circumstantial.

Caswell suggested, based on positional coincidence to 0.5 arc s, that a red object of about 19 mag was identical with the source of radio emission. Inspection of the Palomar Sky Survey shows that the object is diffuse on the red plate, and invisible on the blue plate. The entire region near G127.1+0.5 shows signs of heavy obscuration.

We have made spectrophotometric measurements of the red diffuse object with the image dissector scanner at the 4-m Mayall telescope of Kitt Peak National Observatory. Our observations were obtained on 28–29 August 1978, a night of excellent seeing and transparency. On the slit-viewing television, the object was clearly nonstellar. We integrated for 4,500 s and obtained simultaneous object and sky spectra through two apertures of diameter 3.2 arc s separated by 52 arc s. The net spectrum due to the object covered the wavelength interval 4,000–7,500 Å with a resolution of about 20 Å. Figure 1 shows the distribution of flux density f_λ in erg cm^{-2} s^{-1} Å^{-1} obtained by comparing the counts observed on G127.1+0.5 with those obtained on standard stars of known flux distribution.

As Fig. 1 shows, the spectrum of the object consists of a very red continuum with two broad emission blends. The two blends are at wavelengths of 6,691 Å and 6,841 Å, and have integrated fluxes in the ratio 2.3:1. We identify the stronger, broader feature at 6,691 Å with the familiar blend of [N II] 6,548.1 Å, Hα 6,562.8 Å, and [N II] 6,583.6 Å. The feature observed at 6,841 Å, we identify with the [S II] doublet 6,717.0, 6,731 Å, and we propose that the object is a galaxy.

Fig. 1 Flux density F_λ (erg cm^{-2} s^{-1} Å^{-1}) plotted against wavelength for the diffuse red object at the centre of the supernova remnant G127.1+0.5 ($\alpha = 01$ h 25 min 08.3 s, $\delta = +62° 50' 59''$ (1950)). The two broad emission features at 6,691 Å and at 6,841 Å are identified with [N II]+Hα and with [S II] at a redshift of 5,375±200 km s^{-1}.



Although the individual lines are not resolved by our instrument, we can make a reasonable estimate for the relative line strengths within the blends by using the spectrophotometry of typical radio galaxies compiled by Costero and Osterbrock[5]. Based on their work, we expect that [N II] 6,584 Å will have about the same strength as Hα, and that [N II] 6,548 Å has one-third that strength. We expect that the [S II] 6,717 Å line will have the same strength as the 6,731 Å line. On that basis we compute effective wavelengths for the blends that yield a redshift $cz = 5{,}375 \pm 200$ km s^{-1}. We note that the observed ratio of [N II]+Hα to [S II] is about the same in this object as in the radio galaxies studied by Costero and Osterbrock.

If the object is at a distance of ~100 Mpc, (for $H_0 = 55$ km s^{-1} Mpc^{-1}) then it has an absolute magnitude M_v of about $-16 - A_v$. We can estimate the absorption A_v by noting that the continuum fluxes at 7,000 Å and 5,800 Å are in the ratio 2.7:1. Absolute spectrophotometry by Oke and Sandage[6] indicates that the intrinsic ratio is about 1:1. If we attribute the reddening to dust that follows the Whitford relation[7,8] the required extinction at the visible is $A_v = 5$ mag. Then the absolute magnitude of the object is near $M_v = -21$, the absolute magnitude of a luminous galaxy. The presence of large extinction is corroborated by the low galactic latitude of the source ($b = +0.5°$), the appearance of the region on the Palomar Sky Survey, the absence of the object from the blue plate, and the absence of short wavelength emission lines, such as [O III] 5,007 Å, that are prominent in radio galaxies.

At a distance of 100 Mpc, the radio power of the galaxy integrated to 15 GHz is about 8×10^{40} erg s^{-1}, coming from a region of diameter 0.2 pc. For comparison, the compact source in M 87 has a diameter of 0.04 pc, and about $\frac{1}{4}$ the emitted energy per unit frequency at 15 GHz (ref. 9).

We believe that a completely consistent picture of the central source in G127.1+0.5 is that it is a luminous galaxy at a distance of ~100 Mpc that contains a compact and energetic radio source. Although the circumstantial evidence linking the central source to the supernova remnant is intriguing, we believe that the positional coincidence is mere chance.

Because G127.1+0.5 was the most compelling case for a compact radio source associated with a supernova remnant, the demonstration that this object is a chance event makes it seem likely that other suggested radio objects near supernova remnants may well be just the superposition of background sources on galactic remnants.

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Magnetic field in the primitive solar nebula

METEORITES which fall to Earth's surface generally carry permanent magnetisation which probably was acquired early in their history[1]. In particular, carbonaceous chondrites, which are generally assumed to be virtually unaltered relicts from the earliest Solar System processes of condensation and accretion[2], have apparently been magnetised in magnetic fields with intensities in the range 0.1–10 G (refs 1, 3, 4). The origin of the magnetising field has remained obscure; explanations proposed to account for the field, include a strong central solar field in the early Sun, an intense solar wind magnetic field, magnetic fields generated in large parent objects—ancestral to the meteorites—and the interstellar magnetic field transiently compressed during the Solar System's formation. So far as we know, none of these mechanisms has been shown quantitatively capable of accounting for measured meteoritic remanence[5]. We suggest here that the magnetic field recorded in the remanence of carbonaceous chondrites may have been produced by a self-excited hydromagnetic dynamo in the gaseous preplanetary nebula from which the Solar System is thought to have formed.

The Solar System is generally thought to have formed from the collapse of gaseous interstellar matter which, because of the system's angular momentum, settled into a disk shaped nebula. Several models to describe the evolution of such a nebula have been computed[6–10]. While these models are not unique, they do give some indication of the processes and conditions involved in the early evolution of the Solar System. For specificity, we will use the models recently computed by Cameron[10], which consist of turbulent and differentially rotating, gaseous disks having characteristic radial scales of several astronomical units and characteristics half thicknesses of a few times 10^{12} cm. In the models the gas density characterising the region at several astronomical units from the centre is $\sim 3\times10^{-10}$ g cm^{-3} and the characteristic turbulent fluid velocity is $\sim 4\times10^{4}$ cm s^{-1}.

Turbulent motions in such a differentially rotating gas nebula can generate a large-scale magnetic field through hydromagnetic dynamo action if the electrical conductivity of the gas is sufficiently high. At the low temperatures and high gas densities typical of a preplanetary nebula, the electron density in thermodynamic equilibrium is too low to support hydromagnetic processes. These processes then require nonequilibrium effects to raise the electron density and electrical conductivity. The recent discovery by Lee *et al.*[11] of anomalously large concentrations of ^{26}Mg in some meteorite samples suggests that the short-lived radioactive isotope ^{26}Al may have been present in abundant quantities in the nebular material—the ^{26}Al produced in a nearby supernova thought to have precipitated the Solar System's formation. Cameron and Truran[12] have estimated that ~2% of the Solar System's heavy elements may have been inserted into the nebula by such an event. Ionising radiation from the decay of short-lived isotopes drives up the electrical conductivity of the nebular gas. In conditions which characterise nebular models the conductivity may be so large that the magnetic Reynolds number exceeds unity, thus coupling the behaviour of magnetic fields to the fluid motion.

In a turbulent, differentially rotating gas the efficacy of the fluid motions for generating a magnetic field can be expressed through a dimensionless dynamo number[13,14]

$$N = \gamma\Gamma\delta^3/\eta^2 \qquad (1)$$

where γ measures the shear produced by differential rotation, Γ measures the helicity of the turbulent convection, δ is the physical scale of the magnetic field, and η is the magnetic diffusivity ($\eta = c^2/4\pi\sigma$; c is the speed of light and σ is the electrical conductivity).

Parker has investigated the regenerative modes of a hydromagnetic dynamo operating in a differentially rotating turbulent disk of gas[16] and has found that magnetic fields are produced when $N \gtrsim 6$. It will be shown elsewhere[5] that this condition is satisfied in the model nebula when $\sigma \gtrsim 400$ s^{-1}. Consolmagno and Jokipii[15] suggest that ionisation produced by the decay of ^{26}Al can produce an electrical conductivity of the order of 10^3 s^{-1} in the nebula, thus satisfying the condition for magnetic field generation.

The maximum field intensity that might be realised by the dynamo production process can be estimated. We will consider two dynamical mechanisms that limit the strength of the field. First, because the Coriolis force provides the ordering influence that produces efficient magnetic field generation in the dynamo process, an estimate of the maximum realisable field intensity can be obtained by setting the Coriolis force equal to the Lorentz force produced by the magnetic stress[14]. Thus, in order of magnitude

$$\rho V\Omega \sim \langle B^2\rangle/4\pi\delta \qquad (2)$$

Taking, in equation (2), the density $\rho \simeq 3\times10^{-10}$ g cm^{-3}, the characteristic turbulent velocity $V \simeq 4\times10^{4}$ cm s^{-1}, $\delta \simeq 5\times10^{12}$ cm, the disk scale height, and Ω the Keplerian angular velocity at several astronomical units from the Sun, we find for the characteristic saturation field intensity

$$\langle B^2\rangle^{1/2} \sim 5 \text{ G} \qquad (3)$$

This is the same order of magnitude as the magnetic field which would be necessary to account for meteorite magnetic remanence.

Another mechanism which limits the field strength is ambipolar diffusion, through which the ionised gas and magnetic field slips past the neutral material[17]. Using the results of Consolmagno and Jokipii[15] and equating the ambipolar diffusion time to the turnover time of the turbulent fluid motions one finds that ambipolar diffusion limits the further growth of the magnetic field when the field intensity rises to the order of 1 G.

The values derived here are to a large extent model-dependent and will evolve along with our ideas about the characteristics of a preplanetary nebula. However, these crude and preliminary calculations, taken with the measured remanence of primitive meteorites, suggest that a large-scale magnetic field was generated by gas motions in the preplanetary solar nebula. The intensity of this field may have been as high as 1–10 G.

Such an intense field could have had important dynamical influences on the evolution of the gaseous nebula. It is easy to see that the timescale for redistribution of angular momentum (and thus mass) driven by the magnetic stress, arising from field intensities in the range 1–10 G, is in the range 10^2–10^4 yr. This is the same range estimated for the evolutionary time scale of the primitive solar nebula[10].

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The solar wind influences plasmasphere electron content

MAGNETOSPHERIC plasma convection has been reviewed in detail by Axford[1]. A viscous-like interaction between plasma and magnetic field and magnetic-field-line merging have been suggested as mechanisms producing magnetospheric convection. In either model, convection is sustained by a dawn–dusk electric field (E_{conv}) which has been shown[2–4] to depend on solar-wind speed V_{sw}. Vasyliunas[2] suggested a linear dependence, but Mendillo and Papagiannis[3] estimated that the convection electric field varies nearly quadratically with solar wind speed—this conclusion was supported by Kivelson[4]. The convection field E_{conv} and hence solar wind speed have important roles in determining the location of plasmaspause[5,6]. Model calculations assuming enhancement of the solar wind showed the plasmapause everywhere to be located at smaller L coordinates[7] (L is the McIlwain[8] magnetic shell number). The location of the duskside bulge of the plasmapause has been shown to be inversely related to the solar wind speed[3]. The total plasmasphere electron content (N_P) is supposed to increase with increase in L, the rate of increase being dependent on the density distribution assumed and the L values under consideration[9]. Thus one expects an increase in N_P to be associated with a decrease in V_{sw} and vice versa. In this letter we give an experimental verification of this for two magnetic storms associated with large changes in V_{sw}, and for which N_P and V_{sw} data are available. The results will prove useful in interpreting the disturbed time behaviour of N_P.

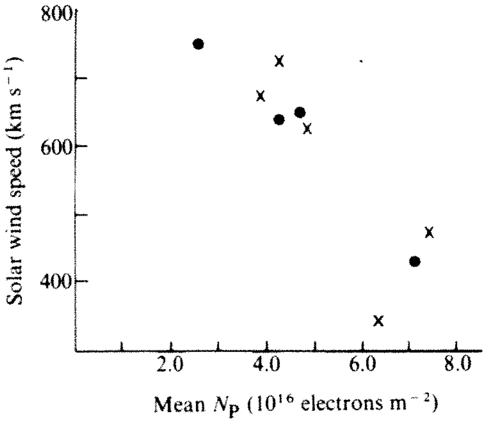

Fig. 1 Solar wind speed plotted against daily mean N_P for the two storms (see Table 1). ●, Storm of 1-5 Nov. 1975; ×, storm of 28 Nov.-2 Dec. 1975.

Electron content measurements were made between October 1975 and July 1976 at Ootacamund (11.4° N, 76.6° E, mag dip 6°) by Faraday rotation and the group delay techniques using radio beacon signals from the geostationary satellite ATS-6 at 35° E. The difference in electron contents measured by these techniques gives the electron content of the plasmasphere[10]. The details of the techniques and the accuracies involved are discussed by Davies *et al.*[11,12]. The daily mean N_P values for the pre-storm and during-storm days are compared with the corresponding solar wind speeds in Table 1. For the first storm the pre-storm solar wind data are not available for the first day. Solar wind speeds are plotted against mean N_P for the corresponding days in Fig. 1. All three pre-storm days have large mean N_P values but low solar wind speeds (~400 km s^{-1}) whereas the during-storm solar wind speeds were greater by a factor of 1.5 with mean N_P correspondingly decreased. This is the first experimental evidence from an equatorial station confirming an inverse relationship between plasmasphere electron content N_P and solar wind speed V_{sw}. The correlation coefficient between N_P and the V_{sw} is -0.92 ± 0.05. A linear relationship between N_P and V_{sw} is obtained by the least square method and is given by $N_P = 12.5(1 - V_{sw}/1000)$ where V_{sw} is the solar wind speed in km s^{-1} ($V_{sw} \leq 750$).

Table 1 Comparison of pre-storm and during-storm plasmasphere electron content and solar wind speed

Storms	MPO	Maximum D_{ST} (γ)	Days	V_{sw} (km s^{-1})	Daily mean $N_P \times 10^{16}$ electrons m^{-2}
1-5 Nov. 1975	2 Nov. 1100 UT	−76	1* Nov.	—	4.5
			2*	430	7.0
			3	650	4.6
			4	750	2.5
			5	640	4.2
28 Nov.– 2 Dec. 1975	29 Nov. 0700 UT	−53	28* Nov.	350	6.7
			29*	475	7.3
			30	625	4.7
			1 Dec.	675	3.8
			2	725	4.2

MPO is the main phase onset; D_{st} is the ring current intensity index.
* Pre-storm days.

Storm-time behaviour of N_P has been discussed by various workers[13–15]. Consideration of solar wind speeds is important in interpreting the observed results and a detailed study of storm-time behaviour of N_P at Ootacamund is underway.

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A new amorphous silicon-based alloy for electronic applications

THERE is a need for alternative energy sources. Photovoltaics are an attractive possibility, but their application has been limited by economic considerations in single-crystal materials, and for physical reasons such as grain boundaries in polycrystalline materials. Amorphous semiconductors are especially attractive in this regard because they are basically much less expensive than their crystalline counterparts and because they possess a direct band gap with a high value for the optical absorption coefficient. We report here the development of a new

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Table 1 Comparison of the new alloy with the best silicone–hydrogen alloy fabricated

Type of amorphous alloy	Si–F–H	Si–H	Comments
Optical band gap E_0	1.65 eV	1.55 eV (refs. 11, 12)	Measured using photoconductive data
Minimum density of localised states $N(E)$	10^{16} cm^{-3} eV^{-1}	10^{17} cm^{-3} eV^{-1}	As measured by the field-effect experiments. The analysis[1] neglects surface states and hence these numbers represent an upper limit to $N(E)$
Intrinsic behaviour	ΔE(~0.8 eV) and σ_{RT} (10^{-10}–10^{-8}(Ωcm)$^{-1}$) are weakly dependent on deposition temperature between 400 and 700 K	ΔE(0.8–0.5 eV) and σ_{RT}(10^{-11}–10^{-7}(Ωcm)$^{-1}$) are strongly dependent on deposition temperature between 350 and 600 K (ref. 13)	σ_{RT} and ΔE refer to the room temperature conductivity and conductivity activation energy
Doping efficiency	Values of $\sigma_{RT} \sim 5$(Ωcm)$^{-1}$ and $\Delta E' \sim 0.05$ eV attained using very small concentrations of PH_3 (500 v.p.p.m.) and AsH_3 (50 v.p.p.m.)	Values of $\sigma_{RT} \sim 10^{-2}$ (Ωcm)$^{-1}$ and $\Delta E \sim 0.20$ eV attained using 4×10^3 v.p.p.m. PH_3 in SiH_4	$\Delta E'$ refers to an estimate of the activation energy at room temperature by drawing a tangent to the σ–T curve
Mode of deposition	Radio frequency glow discharge in SiF_4/H_2	Radio frequency glow discharge in SiH_4 gas	
Photostructural changes	Absent	Major[6]	
Photoconductivity σ_L	(1–3)10^{-4}(Ωcm)$^{-1}$	(1–3)10^{-4}(Ω cm)$^{-1}$ (ref. 14)	Measured under AM − 1 incident radiation of power 90 mW cm^{-2}
Infrared peaks			Thick deposits (~5 μm) made on crystalline Si substrates
2,100 cm^{-1}	Very small	Present	Usually atributed to SiH_2 (ref. 15)
2,000 cm^{-1}	Absent	Present	Usually attributed to Si–H stretch[15]
1,000 cm^{-1}	Present	Present	Usually attributed to Si–O stretch[15]
900 cm^{-1}	Absent	Present	Usually attributed to H–Si–H scissors[15]
830 cm^{-1}	Present	Absent	Attributed to Si–F stretch[11]
640 cm^{-1}	Present	Present	Attributed to Si–H wag[15]
Hydrogen content	~0.5%	>5%	Estimated from the integrated absorption coefficient by using infrared techniques[16]
Fluorine content	~2 to 6% dependent on ratio of SiF_4/H_2	None	Estimated from ESCA measurements

alloy that eliminates the physical problems associated with the silicon–hydrogen alloys.

Amorphous semiconductors ordinarily have a large density of localised states which act as traps which lead to low values for the drift mobility and the recombination time of free carriers. For example, elemental amorphous silicon is of little technological importance because it contains a density of localised states in excess of 10^{20} cm^{-3} eV^{-1}. Madan *et al.*[1,2] reported that amorphous-silicon films decomposed from silane gas by an r.f. glow discharge and deposited on a heated substrate have a much reduced density of states at the Fermi level, of the order of 10^{17} cm^{-3} eV^{-1}. This material has subsequently been shown to be an amorphous silicon–hydrogen alloy[3]. In addition, Spear and Le Comber[4] showed that this alloy could be doped with phosphorous and boron; Carlson and Wronski[5] further demonstrated that it had a sufficiently high carrier lifetime to yield solar cells of up to 5.5% efficiency. Nevertheless, the still relatively high density of localised states throughout the gap leads to inefficient doping and limits the carrier lifetime. In addition, undesirable photostructural changes have been observed[6] and the material effuses hydrogen above 450 °C (ref. 3). Despite many efforts, there has been no success in overcoming these basic problems.

The new alloy has silicon and fluorine as its main structural components. This alloy is multi-elemental and includes hydrogen, and can also include other elements such as oxygen without deleterious effects. The steric chemical and electrical properties associated with our new alloys will be described elsewhere[7,8]. Table 1, which condenses data taken from more than 600 sample depositions, compares our Si–F–H alloy (fabricated from the gas ratio of $SiF_4/H_2 = 10/1$) with the best Si–H alloy.

Table 1 shows that our material is in almost all properties, except for photoconductivity, distinctly different from the best Si–H alloy which makes it attractive for electronic device applications. Of special interest is the fact that the intrinsic properties of our material are only weakly dependent on the substrate temperature, much like multi-component chalcogenide glasses, and in sharp distinction from the strong dependence observed in silane-decomposed films.

With regard to the infrared spectrum, it is important that the peak at 2,000 cm^{-1} corresponding to the silicon–hydrogen stretch does not appear in our films. The peak at 2,100 cm^{-1} is ordinarily assigned to the SiH_2 group which has a wag at 640 cm^{-1} and a H–Si–H scissors mode at 900 cm^{-1}. The scissors mode is also absent in our film. Hydrogen through its various bonding configurations with other elements can be responsible for localised states in the gap[9,10]. A full interpretation of this point and the above data will be given elsewhere[11].

The new alloy reported here is superior to silane-based films for electronic applications. In particular, there is a much lower density of states at the Fermi level. This leads to much larger doping efficiencies. In fact, effective n-type doping has been achieved with only 50 v.p.p.m. AsH_3 in the premix. Furthermore, no photostructural effects have been observed. Because of its highly desirable characteristics, this material seems to have a potential for general semiconducting applications as well as for photovoltaics.

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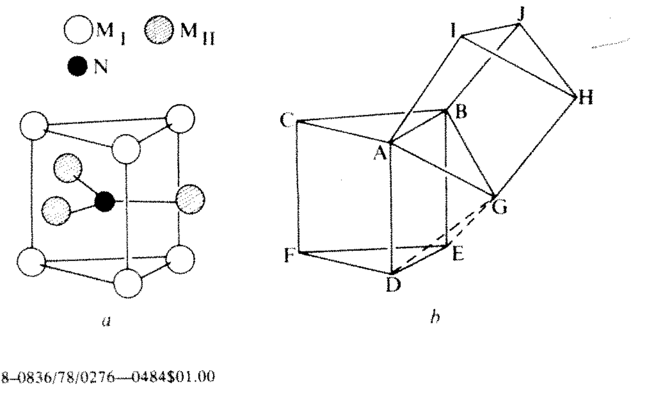
A new structural model for transition metal–metalloid glasses

CONCEPTUAL notions of the structure of metallic alloy glasses have generally developed from purely geometrical sphere-packing models proposed for liquid[1] and glassy[2,3] monoatomic metals. The assumption on which these models are based is that the glass consists of a random arrangement of spherical atoms of each element—the so-called dense-random-packed (DRP) model. Glassy 'metallic' alloys of two or more chemically dissimilar elements can also be represented in this way, local atomic arrangements arising by chance, constrained only by geometrical factors such as the ratios of atomic radii. Construction of the model described here is based on an alternative principle commonly observed in amorphous solids of other types, namely that local structure is well-defined and almost always identical to that found in crystalline forms of the material. The properties of such a 'stereochemically designed' model have been computed and are found to be in reasonable agreement with high resolution neutron scattering data for glassy palladium–silicon alloys.

Metallic alloy glasses can be subdivided into two broad categories: alloys of two or more metallic elements (metal–metal glasses) and those systems in which a non-metallic or semimetallic (metalloid) element is essential for glass formation. Within the latter category, the binary and ternary alloys of the transition metals, particularly Fe, Co, Ni, Pd and the non-metallic elements B, C, P and Si comprise the most important series, commercially and scientifically. Dense-random-packed structures for these glasses allow the non-metallic atom to occupy polyhedral cavities of several different types, only one of which resembles the local coordination observed in the crystalline state. Structures represented by this model are essentially different from those of most other types of glass: in oxides and chalcogenides, amorphous elemental and compound semiconductors, organic 'molecular' and polymeric glasses, the nearest-neighbour coordination and local symmetry, at least, are well defined. It is clearly incorrect, for example, to represent a glassy silicate as anything other than a non-periodic arrangement of relatively undistorted SiO$_4$ tetrahedra[4,5], the latter being, of course, the local structural units on which nearly all simple silicate lattices are based.

Fig. 1 *a*, Regular trigonal prismatic coordination; and *b*, edge-sharing of polyhedra observed in the Fe$_3$C, cementite structure. Note that the M$_\mathrm{I}$ atom G for the upper prism occupies an M$_\mathrm{II}$ site in relation to the lower.



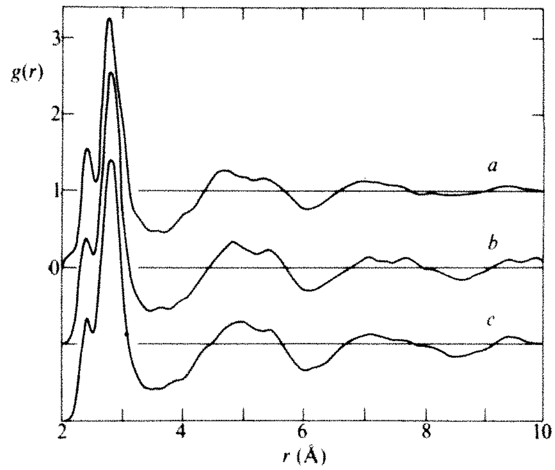


Fig. 2 Pair correlation function $\rho(r)/\rho_0$ for glassy Pd$_4$Si, where $\rho(r)$ is the atomic density and ρ_0 the average density. *a*, Experimental data derived from neutron scattering measurements[12] with $Q_{\max} = 25$ Å^{-1} ($Q = 4\pi \sin\theta/\lambda$). Curves *b* and *c* refer to spherical cores of the model of radius 8.6 and 10 Å respectively. The latter cluster is not fully dense but nonetheless gives a better representation of $g(r)$ beyond ~5 Å where the effects of finite model size become important. Computed curves have been convoluted with gaussian broadening functions to simulate the effects of thermal vibration and transformation of experimental scattering data over a limited range of Q. Values were chosen to reproduce the breadth of each component of the split first peak at 2.4–2.9 Å (half widths are 0.094 Å for Si-Pd and Si-Si, and 0.14 Å for Pd–Pd correlations).

Local structure in the borides, carbides, phosphides and silicides of most of the transition metals which form glasses on quenching from the melt is also well defined in the crystalline state[6]. The coordination polyhedron of the non-metallic element, N, consists of a subshell of six metal atoms, M$_\mathrm{I}$, forming a somewhat distorted trigonal prism with three further M$_\mathrm{II}$ atoms capping the square faces at somewhat larger and even more variable M–N distances (Fig. 1*a*). Do the glassy forms of these materials therefore also consist of non-periodic arrangements of such units? Are glasses, formed from palladium and silicon or iron and boron, random amorphous alloys of the two pairs of elements, or glassy silicides and borides with well defined local symmetry similar to that found in the crystalline forms?

The influence of localised, possibly directional chemical bonding, the formation of relatively large groups of atoms with low symmetry, are relevant factors—if only in a qualitative sense—to theories of glass formation and stability. Arrangements of spherical atoms into a periodic array involves relatively simple processes, whereas ordered packing of large assymetric 'molecular' fragments requires a series of correlated motions. Glass formation is therefore more likely in the latter case, as has been noted elsewhere[7]. Alternative theories stress the stability gained when atoms of one element fit within cavities formed by random packing of the other[8] or collective properties of the valence electrons—specifically, the reciprocal space relationship between the Fermi momentum and the first peak of the structure factor[9]. In neither of the latter treatments is bonding considered explicitly. The importance of each of these three factors in particular alloy systems is a source of controversy[10].

This paper describes properties of an atomic model for metal–metalloid glasses in which trigonal prismatic NM$_6$ units are connected in an essentially random manner, with an edge-sharing arrangement (Fig. 1*b*) observed in many of the crystalline forms of these materials, notably the Fe$_3$C cementite structure. A physical model, built from 3-cm polystyrene spheres, was used to guide a computer program which generated the atomic coordinates of a cluster with a radius of about 12 Å containing 434 M and 108 N atoms. Atomic coordinates are unrealistic at this stage for two reasons. First, regular, undistorted trigonal prisms were used to simplify the construction algorithm; however, N atoms are generally too large to fit within

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a regular trigonal prism which must therefore distort. Second, the physical model allows no positional adjustment once units are fixed in place and it is impossible therefore to build a model which does not contain relatively large voids on the one hand and 'compressed' atoms on the other, although excessive overlap of atoms can be avoided in both the hand-built and computer-generated models. Coordinates were refined by moving atoms sequentially to minimise the energy calculated using a Lennard–Jones pair potential

$$V_i = 4\varepsilon_{ij}\sum_j (R_{ij}^{12} - R_{ij}^{6})$$

Here, V_i is the energy of atom i, $R_{ij} = 2^{-1/6}\,R_{ij}^0/r_{ij}$, where r_{ij} is the actual distance between the atoms i and j and R_{ij}^0 the eqilibrium distance. The quantities ε_{ij} are approximately proportional to weighted sums of the cohesive energies of each element and have been obtained from the tabulation of Gschneider[11]. In the summation, j includes all nearest-neighbour atoms: for M–M pairs, atoms within a sphere of radius 3.2 R_M^0 centred on the origin atom are included where R_M^0 is the radius of the metal atom. Atoms are free to move in and out of this sphere as energy minimisation proceeds. For N–M pairs, however, trigonal prismatic local coordination is emphasised by attempting to keep the local environment of the N atoms invariant, the program attempts to retain the original six nearest-neighbour M neighbours of each N atom throughout the calculation. Moreover, the average bond length is set to the N–M_I distance found in crystals. Further M_{II} metal neighbours lying with 2.4 R_M^0 are included in the summation: these atoms are unconstrained and a larger equilibrium N–M_{II} distance is assumed.

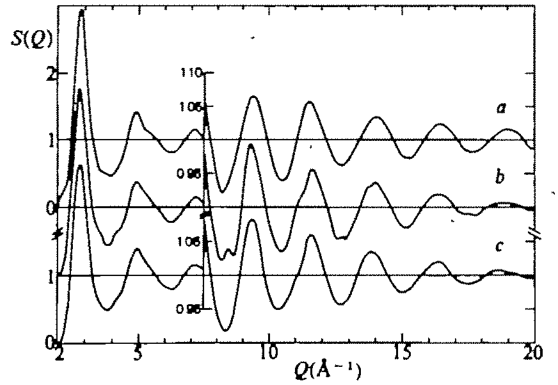

Fig. 3 Neutron structure factors $S(Q)$ for a–Pd_4Si: labelling as for Fig. 2. The computed curves were obtained by a sine transform of real-space data (over the range 0–15 Å) modified by a weighting function sin $(\pi r/12)/(\pi r/12)$ introduced to suppress errors in the radial distribution function beyond about 8 Å.

Figure 2 shows the pair correlation function $g(r) = \rho(r)/\rho_0$ computed for glassy Pd_4Si, compared with experimental data derived from neutron scattering measurements[9]. Here, $\rho(r)$ is the atomic radial density function and ρ_0 the average atomic density. The function $g(r)$ has been calculated for spherical cores of radius 8.6 and 10 Å. As the model is small, the average density is a function of the cluster radius—a core of radius 8.6 Å is almost fully dense whereas at 10 Å, parts of the spherical core lie beyond the edge of the model. The average density, 10.4 ± 0.2 g cm^{-3} is in good agreement with experimental values for a-Pd_4Si of 10.25 (ref. 3) and 10.5 g cm^{-3} (Suzuki, personal communication). The neutron scattering factor, $S(Q) = 1 + 1/Q \int_0^\infty 4\pi r(\rho(r) - \rho_0)\sin Qr\,dr$ is shown in Fig. 3.

Although there are some discrepancies between computed and experimental $g(r)$ values—notably the height of the first (Si–Pd) peak at 2.4 Å and the position of the first component of the second split peak at 4.8 Å, overall agreement is reasonable. Agreement between experimental and computed neutron scattering data, Fig. 3, is excellent, the somewhat more rapid decay of oscillations in $S(Q)$ for the model beyond about 17 Å^{-1} indicating greater order in the real glass.

This work cannot conclusively resolve the issue of defined (chemical) compared with random (geometrical) local structure, scattering data are almost certainly insufficiently sensitive to discriminate between the two models. However, the defined local coordination model apparently gives a good representation of transition metal–metalloid glasses, especially those which have been quenched relatively slowly or have been subsequently annealed and thus approximate to the minimum free energy amorphous state–an 'idealised' glassy state–the vitreous equivalent of the perfect single crystal. In this context it is interesting that atomic coordinates can be generated for a model in which the local coordination around silicon atoms is less well defined (by removing the distinction between N–M_I and N–M_{II} distances mentioned above). Changes in $S(Q)$ thus introduced give a good representation of experimental changes in X-ray scattering observed when metallic glasses are annealed at temperatures below their glass transition or crystallisation temperatures[13,14], the more ordered model corresponding most closely to an annealed glass.

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Transient effects in laboratory measurements of compressional wave velocity and electrical conductivity

LEE *et al.*[1] demonstrated the effect of temperature on the electrical conductivity of high grade saturated metamorphic rocks, when held at constant effective pressures. Apart from initially showing an increase in conductivity with temperature, it was demonstrated that conductivity at higher temperatures was time dependent. When a sample was held at a constant temperature (~250 °C) the conductivity decreased exponentially with time, so that after ~10 h, there was little further change observed. As the temperature was lowered the conductivity decreased from this steady state value, and with further thermal recycling the conductivity was recycled along a path

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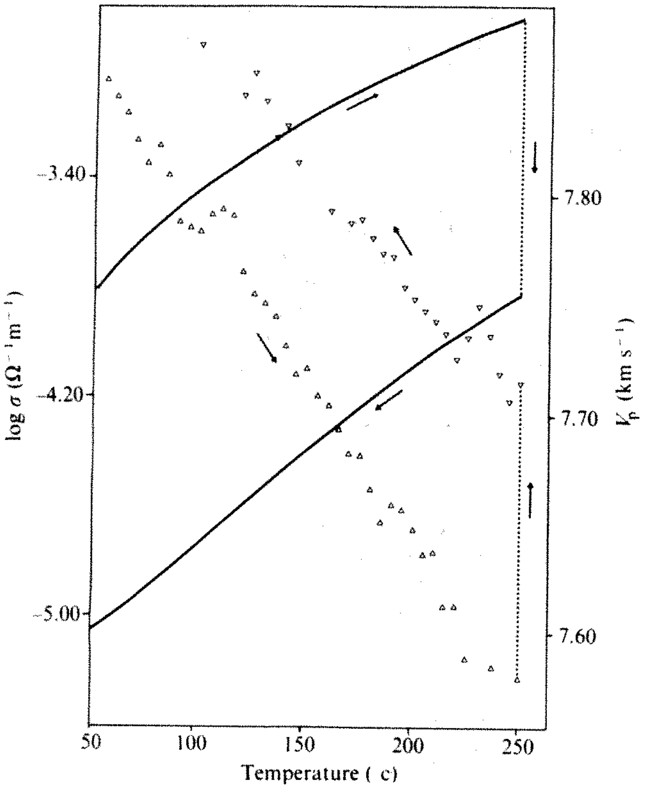

Fig. 1 Electrical conductivity and compressional wave velocities for a saturated (0.5 M NaCl) sample of ultramafic rock as a function of temperature. The electrical conductivity is described by the solid line and compressional velocity by triangles, inverted for decreasing temperature. The transient effect at elevated temperatures is signified by dotted lines. Confining pressure, 0.35 GPa; pore pressure, 0.10 GPa.

uite different from that taken with the initial temperature icrease. The results implied that due regard should be paid to iis transient effect when making laboratory electrical ieasurements on rock samples in simulated upper crustal onditions. Time-dependent effects at elevated temperatures ave also been observed in laboratory measurements of ompressional wave velocities[2,3]. Spencer and Nur[2] in their udy of the Westerly Granite showed that thermal recycling at evated pressures tended to produce an irreversible increase in ompressional velocities. Further investigation demonstrated a ystematic time-dependent increase of velocity while the sample was held at a fixed elevated temperature (495 °C). We report here the direct comparison of these transient variations of electrical conductivity and compressional velocity. In our experiments we have measured these parameters simultaneously on the same sample to ensure that any variables such as pore pressure, confining pressure and temperature are identical. Any comparison of separate measurements of conductivity and velocity on the same sample in different experiments is unsatisfactory due to the irreversible nature of the process being studied. Even if two samples from the same rock are used there may be significant differences in cracks and mineralogy, which would give erroneous results.

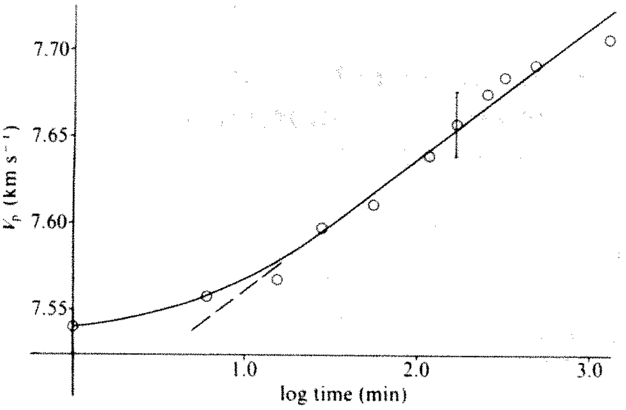

Fig. 2 Compressional velocity as a function of log time for a saturated (0.5 M. NaCl) sample of ultramafic rock while held at 250 °C. Confining pressure, 0.35 GPa; pore pressure, 0.10 GPa.

The apparatus we used is similar to that of Lee *et al*[1], except for the introduction of lithium niobate transducers within the metal electrodes. A pulse transmission method was used[2], with a 60 V (0.5 mHz) square wave applied to the input transducer and the resultant output signal (received by the output transducer) displayed on a dual-beam oscilloscope. The transit time through the sample was measured by a Hewlett-Packard timer counter triggered by the input pulse and terminated by a marker pulse, visually aligned with the first arrival of the output signal. This method introduced an error of ~0.5% between different measurements and an absolute error of ~3% in velocity readings.

A wide range of rocks including granite, quartzite, gneiss, schist, gabbro and basalt have been studied at elevated pressures and temperatures (confining pressure 0.35 GPa; pore pressure 0.1 GPa; temperature up to 250 °C). All have shown the transient decrease in conductivity described by Lee *et al.*[1], and with this a corresponding velocity increase.

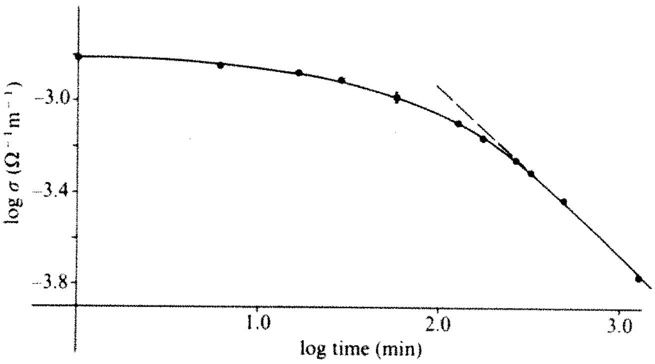

Fig. 3 Log conductivity as a function of log time for the same sample of ultramafic rock as that described in Fig. 2. The measurements of conductivity were made simultaneously with the velocity. Temperature, 250 °C; confining pressure, 0.35 GPa; pore pressure, 0.10 GPa.

A clear example of these effects are shown in Fig. 1, which depicts the first thermal cycle on a sample of ultramafic rock. While the sample was held at 250 °C there was an initial rapid increase in velocity with time ($\dot{V}_p \simeq 4 \times 10^{-4}$ km s^{-1}/min). The rate of increase in velocity, $\dot{V}_p$, decreased inversely with time so that after ~48 h a steady state maximum value of velocity was obtained. After the initial increase a linear relationship is suggested between the compressional wave velocity and log time (in min) (Fig. 2): $V_p \propto \log$ (time).

The results of the corresponding conductivity measurements are shown in Fig. 3. Lee *et al.* suggested that after the initial drop in conductivity (where uncertainty arises about the precise onset time): $\log \sigma \propto \log$ (time).

One might expect therefore a close relationship between the change in compressional wave velocity and the change in electrical conductivity. However, a direct comparison of these two parameters is unsatisfactory, due largely to the magnitude of the increase in velocity at the later stage of the transient effect being small compared to the observational error. In any case, Figs 2

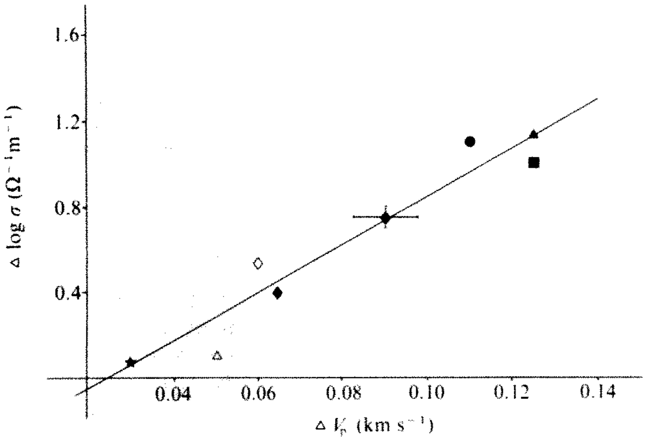

Fig. 4 Comparison of the magnitude of the transient compressional wave velocity and log conductivity effects over a period of 17 h for several rock types. Temperature, 250 °C; confining pressure, 0.30 GPa; pore pressure, 0.10 GPa; pore fluid, 0.5 M NaCl. ▲, Saturated granite; △, dry granite; ■, peridotite; ●, garnet mica schist; ◆, gabbro; ◇, gneiss; ★, quartzite.

and 3 suggest that the relationship may be more complex, in that the onset times of the linear segments are quite different for the two parameters. Comparison of the magnitude of the transient effect for several rock types and over a fixed time interval (Fig. 4) does show, however, a general relationship between the change in velocity and the change in (log) conductivity.

Spencer and Nur[2] suggested that the variation in velocity with time might be due to a solution of quartz in supercritical pore water. An increased content of silica in the pore fluid might increase the bulk modulus or viscosity of the fluid and hence the compressional velocity in the aggregate. However, increased silica content would also increase the electrical conductivity[6], whereas our observations of the transient effect associate increasing velocity with decreasing conductivity.

Lee *et al.*[1] suggested that the transient behaviour of electrical conductivity might be a sensitive indicator of a creep process. A primary creep[4] mechanism at these elevated temperatures and high effective pressures would result in the further closure of low aspect ratio microcracks, resulting in a significant drop in electrical conductivity[6]. It would also produce a marked decrease in compressibility of the sample[5] giving an increase in compressional wave velocity.

Our results lead us to believe that elevation of temperatures above 250 °C at a high effective pressure produces an irreversible process within the samples. This process must affect a large group of physical parameters and consequently be important in any discussion of experimental work when upper crustal conditions are simulated. In particular, measurements of temperature effects on conductivity and velocities should require a thorough thermal recycling study.

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Nuclear magnetic resonance spectroscopy of humic materials

ALTHOUGH humic substances are some of the most important naturally occurring macromolecules in both soil and aqueous environments, their structures are still unknown. Our understanding of the components which comprise humic mixtures has been confined to the analysis of only minor portions (5–10%) of the total humic entity. It is, however, the whole humic aggregate, most of which is structurally unknown, which plays an active nutritional or transport role in nature. When the major fraction of humic matter, the alkali soluble substances, are studied, only poorly defined spectra have been obtained. Infrared, ^{1}H-NMR and ^{13}C-NMR spectroscopic techniques have yielded unresolved spectra in which little detail can be distinguished[1–5]. We report here that, using large magnetic fields and very high frequency (270 MHz) Fourier transform ^{1}H-NMR instrumentation, we have found that a wealth of detail can be obtained on the structure of humic solutions. A convolution difference method[6] allows discrete signals to be detected above the general broad band absorptions in much the same way as important structural and dynamic information concerning proteins has been gathered.

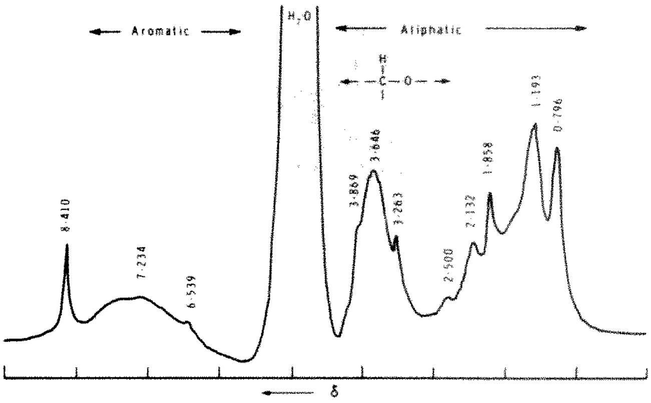

Fig. 1 270 MHz ^{1}H-NMR spectrum of Wakanui humic material in NaOH; chemical shifts are in p.p.m from internal TMS. They were measured with respect to external TMS and corrected. A pulse of 10 μs (85° tilt angle) was used to view a spectral window of 3.6 kHz in 8 K data points. Humic material was prepared by extraction of soil with 0.1 mol dm^{-3} NaOH. The mixture was acidified and the precipitate redissolved in NaOH for spectral examination.

The 270 MHz Fourier transform proton spectra of alkali soluble Wakanui silt loam (Wakanui silt loam—parent material—late Pleistocene loess imperfectly drained with seasonal high water table. pH = 5.8; C% 4.0, N% 0.35; C/N = 11.4, C.E.C. (me%) 23.4. New Zealand classification—yellow-grey earth) 'A' horizon material is shown in Fig. 1. The peak at 8.4δ arises from formate ion. This is confirmed by the signal at 167δ in the ^{13}C-NMR spectrum. From the integrated spectrum it is possible to estimate the fraction of protons, excluding exchangeable protons, which are aromatic (H^{*}_{ar}), and the fraction which is aliphatic (H^{*}_{ali}).

The Brown and Ladner equation[7], $f_a = [(C/H) - 0.5H^{*}_{ali}](C/H)^{-1}$, (where C/H is the carbon to hydrogen mol ratio), has been successfully applied to a range of carbonaceous mixtures for measuring the fraction of aromatic to total carbon (f_a) in the mixture. Recently, its basic assumption that the mol ratio of aliphatic hydrogen to aliphatic carbon is of the order of two, has been confirmed by ^{13}C-NMR spectroscopy[8]. ^{13}C-NMR spectroscopy of humic substances is still in its infancy[5] and when applied the Brown and Ladner assumptions may not be valid because of the predominance of COOH groups in humic substances. However, if f_a is redefined as f'_a, the fraction of aromatic carbon plus carboxylic carbon to total carbon, it becomes a useful parameter for describing the structure of humic substances.

Our results indicate that 65% of the carbon in Wakanui silt loam extract is aromatic and carboxylic acid carbon. The parameters f'_a and H^*_{ar} are 0.65 and 0.16 respectively. For moss peat soil, H^*_{ar} is around the same value but f'_a is much lower (0.50). This contrasts with a podsol 'A' horizon of $H^*_{ar} = 0.43$, $f'_a = 0.72$. Use of Brown and Ladner's parameters, σ, the degree of substitution, and H_{aru}/C_{ar}, the atomic hydrogen to carbon ratio of the hypothetically unsubstituted aromatic material is not justified because large amounts of oxygen-bonded aliphatic material are present in our extracts.

The combination of a low H^*_{ar} and high f'_a value recorded for loam extract, indicates that the solution contains aromatic material which is polycyclic or heavily substituted monocyclic. The presence of aromatic ^{1}H-NMR absorptions at >8.1 δ can be assigned to sterically hindered protons characteristic of many polycyclics. In our spectra they have only a very minor contribution to the total aromatic absorption.

From the combustion analysis, we were able to show that the mol ratio of hydrogen to nitrogen in this substrate is 12.4. In view of the known forms of nitrogen in humic materials[1], most of the nitrogen is probably bound to one or less protons. The contribution of nitrogen-bonded protons must then be $\leq 8\%$.

Of the rest, it seems from the absorptions around 3.6 δ that a large proportion (32%) of the aliphatic, or 27% of the total (excluding exchangeable) protons, is bound to carbon α to oxygen. The predominance of such aliphatic oxygen-bound carbon suggests a major proportion of sugar-like components or other polyhydroxy or polyether materials. This is supported by the ^{13}C-NMR spectrum (Fig. 2*c*) which also indicates COOH, aromatic and alkyl groups[5].

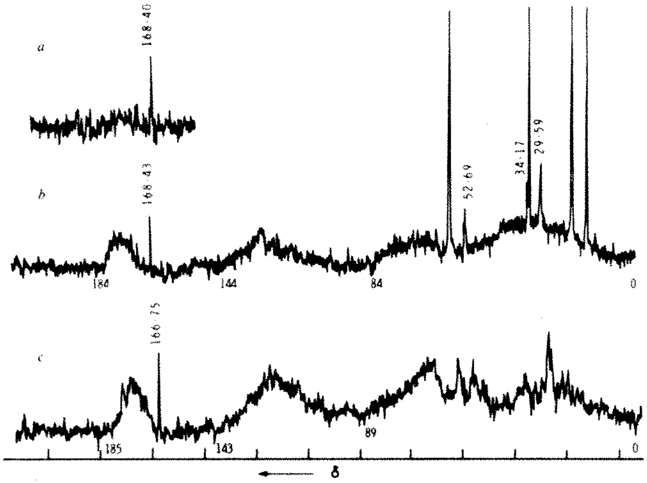

Fig. 2 ^{13}C NMR spectra of humic materials: *a*, Mangaroa humic acid peak at 168 δ; *b*, Mangaroa fulvic acid; *c*, Wakanui humic acid. Signals were observed relative to external TMS and corrected relative to internal TMS. Observed signals for Wakanui material were: 179.69, 184–169 (broad halfwidth = 9, δ_{max} = 177.06, COOH) 166.75 (strong), 143–106 (broad halfwidth = 17, δ_{max} = 127.02, aryl) 84–65 (broad halfwidth = 13, δ_{max} = 70.27, RZHCOZ, Z = R, H) 59.71, 59.3, 54.35, 35.80, 27.58 (strong, CH_2) 26.61, 22.6, 20.4. Observed signals for Mangaroa fulvic acid were 185–171 (broad, halfwidth = 8, δ_{max} = 181.24) 168.43 (strong), 144–104 (broad halfwidth = 10, δ_{max} = 128.82) 89–63 (broad, halfwidth = 16, δ_{max} = 74.54) 52.69 (strong), 34.17 (strong), 29.59 (strong) 55–8 (broad). 1-butanol peaks have not been listed. Spectra were recorded at 67.89 MHz with a 22 μs pulse (60° tilt angle). Acquisition time was 1.08 s for a 15 kHz spectral window in 32 K of memory and a delay of 1–2 s. Data were accumulated over 12 h.

Our spectra confirm the previous results[4,9–12] that alkyl chains make important contributions to humic substances. The signal at around 0.796 δ can be ascribed to CH_3 γ or further from aromatic rings. Polymethylene is present probably because of its low biodegradability[9].

In humic materials isolated from river sources, we observed the same general patterns although the $\mathrm{H{-}\overset{|}{\underset{|}{C}}{-}O{-}}$ absorptions were not as distinct, and protonated aromatic material represented only a very minor contribution. We were able to show by attenuation that the fine structure of the aromatic absorptions from both potable (Akatawara) and swamp water (Mangaroa) sources were very similar (see legend to Fig. 3 for a description of the waters). Application of a resolution-enhancement technique[6] which uses the difference between the broadened and resolved signals revealed detailed resonance information (Fig. 3). For example, Mangaroa humic acid isolated from a typical swamp water source in New Zealand showed at least six clear-cut aliphatic resonances which could be followed by, say, chemical modification of the humic material. Similarly, fine structure was found for samples from a potable (Akatawara) aqueous source.

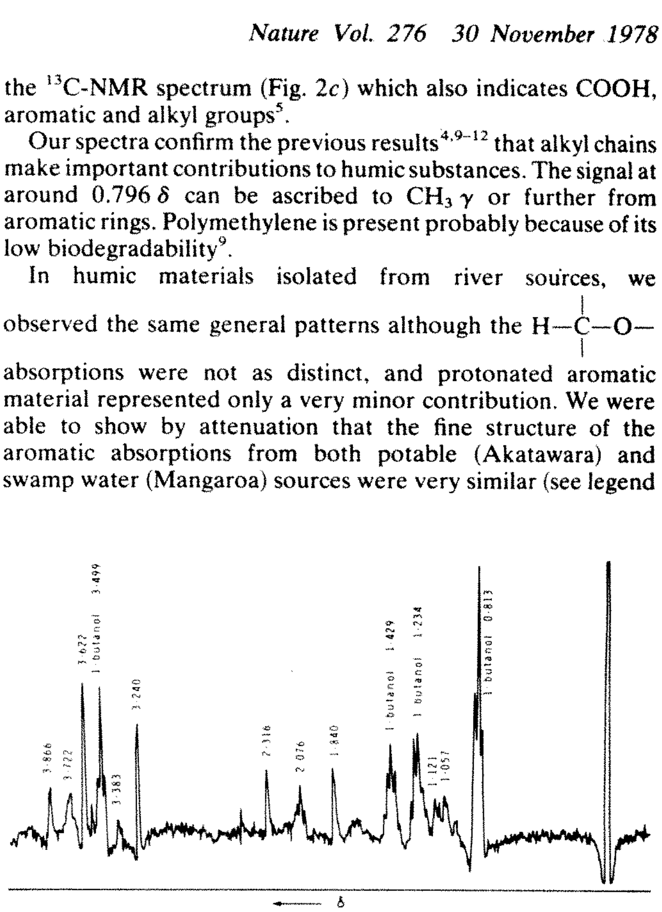

Fig. 3 Convolution difference spectrum of Mangaroa humic material. The signals due to 1-butanol arise from solvent tightly bound to the humic material which we were unable to remove on drying to constant weight. Samples were prepared by common water chemistry techniques[15] involving filtration, concentration, acidification and extraction of 1-butanol-soluble material. Mangaroa humic material: source—Black Creek, Mangaroa Valley, New Zealand. $pH = 6.5$; conductivity, 8.0 mS m^{-1}; absorbance, 0.990 at 270 nm; colour, 300 Hazen units. Akatawara humic material: source—Akatawara River. Upper Hutt, New Zealand (typical potable water). $pH = 6.9$; conductivity 7.1 mS m^{-1}; absorbance 0.210 at 270 nm; colour 40 Hazen units.

Our results agree with the increasing amount of evidence[4,5,9,13] that humic materials are not coal or lignin like in structure. The presence of large amounts of aliphatic polyhydroxy groups explains both the insolubility of humic materials in organic solvents and the behaviour of these substances to methylation[1,14]. Of particular interest is a methylated chloroform-soluble subfraction of a fulvic acid isolated by Schnitzer[10]; this has similar important absorptions around 3.6 δ. The subfraction has a number average molecular weight of 821 g mol^{-1} and possibly represents a small fragment of humic material of a type not unlike some of the macromolecules observed in our extracts. Some of the signals may not arise from methylation.

In contrast to other branches of environmental science, structural studies on soil and aqueous organic matter have always been hindered by a lack of available analytical tools. Our results reported briefly here show that at last this tool has arrived and that the next decade will see a great increase in studies using

NMR techniques which will throw new light on the chemistry of these elusive materials.

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Desert varnish: evidence for cyclic deposition of manganese

WE have found evidence that desert-varnish coatings on rocks in the western US consist of micron-scale alternating dark and light layers that differ in manganese and iron content. We report here the first quantitative electron-microprobe analyses of desert varnish, that also show the chemical variations in these layers. In the field and laboratory we obtained new evidence that varnish covers a fresh substrate by nucleation of micron-sized patches that grow and coalesce.

Varnish-coated samples from Arizona, Utah and Idaho were studied in the field and in the laboratory. Host rocks include basalt, granite, quartzite, limestone, sandstone, caliche, quartz and opal. Varnish coatings range in thickness from a few micrometres to hundreds of micrometres, and characteristically are rich in manganese and iron[1-13]. Varnishes from widely separated localities have similar chemical, mineralogical, morphological and optical properties, and are compositionally independent of the host rock.

Ultra-thin (~10 μm) sections cut normal to the rock surfaces were prepared (by us and by R. Beauchamp). Sections from the three localities reveal alternating black and light-red layers that range in thickness from 0.25 to ≳20 μm (Fig. 1). The layers are typically a few micrometres thick. Mound-like or botryoidal structures are common, and within these structures the thicknesses of the layers are highly variable. Many layers can be traced laterally for at least several millimetres, except within the botyroidal structures where layers may extend for only tens of micrometres. Sections from single boulders show correlations between major layers in the varnish. Because of the difficulty of preparing ultra-thin sections (without losing the soft varnish) we have insufficient samples to say whether varnish layers can be correlated among samples on a local or regional scale.

Representative electron-microprobe analyses of varnish layers are given in Table 1. The areas analysed are shown in Fig. 1*a*. An analysis of the feldspathic matrix of the basalt substrate below the varnish is included in Table 1. Although it has long been known that desert varnish is rich in manganese[12,13] our data show that the manganese is concentrated in the optically opaque or dark layers (~20 wt% MnO_2) and that the adjacent red layers are relatively Mn-poor (~3 wt% MnO_2). The red layers are enriched in Fe_2O_3, SiO_2, Al_2O_3 and K_2O, whereas the dark layers contain more CaO. Other elements such as Ti, Mg, Na, P and S seem to be fairly evenly distributed throughout the coating.

In thin sections we can distinguish optically between the layered varnish and pockets of detrital material (Fig. 1*b*) consisting largely of clay with minor amounts of feldspar, quartz and hematite. The detritus typically makes up <30% of the coating. It occurs between botryoidal mounds where it seems to have been trapped by the growing varnish. In Fig. 2 the scanning electron microscope shows the botryoidal surface of the same varnish sample in Fig. 1. Figure 2 also shows random detrital minerals, and illustrates how grains can accumulate between the mound-like structures. These morphological relationships imply that analyses of scrapings of desert varnish include both layered varnish and trapped detritus.

The mineralogy of the varnish is not yet completely understood. Potter and Rossman[2-4] using scrapings of varnish from the Mohave Desert, California, concluded on the basis of mid-

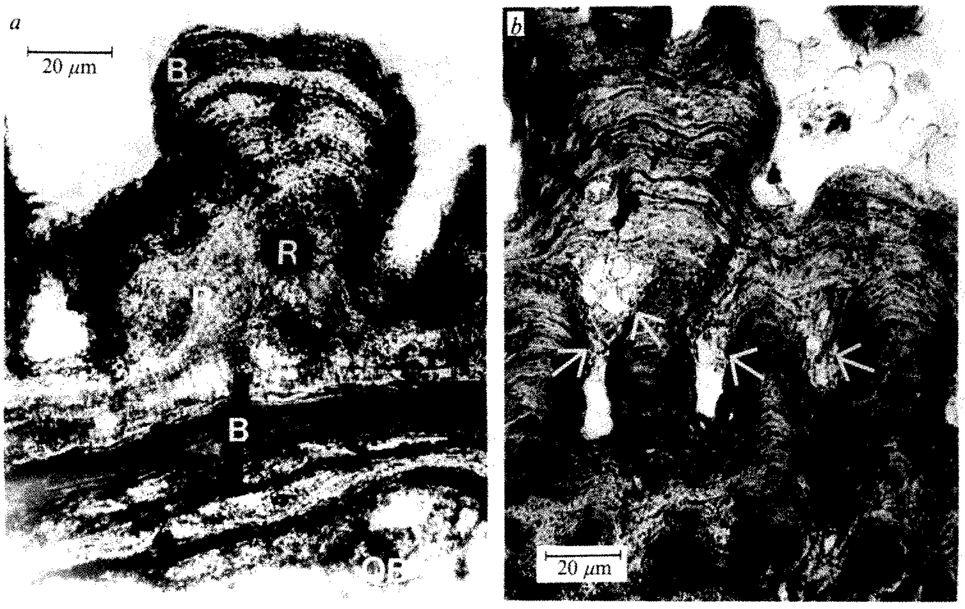

Fig. 1 *a*, Photomicrograph of Arizona desert varnish in a thin section cut normal to the surface of the sample. The varnish coats an olivine basalt (OB). Dark (black) (B) and light (red) (R) layers express mainly variations in Mn/Fe ratio (see Table 1) and imply alternating conditions of deposition. Mound-like structures are similar to those in Fig. 2. The closely spaced black bands at B are where electron microprobe analyses were made. (R) marks analyses representing an Mn-depleted and Fe-rich zone. The dark spot and lines are artefacts from the electron microprobe beam. Plane polarised light. *b*, Similar to (*a*) Arrows indicate pockets of detrital clays with minor feldspar, zeolite (?), quartz and hematite.

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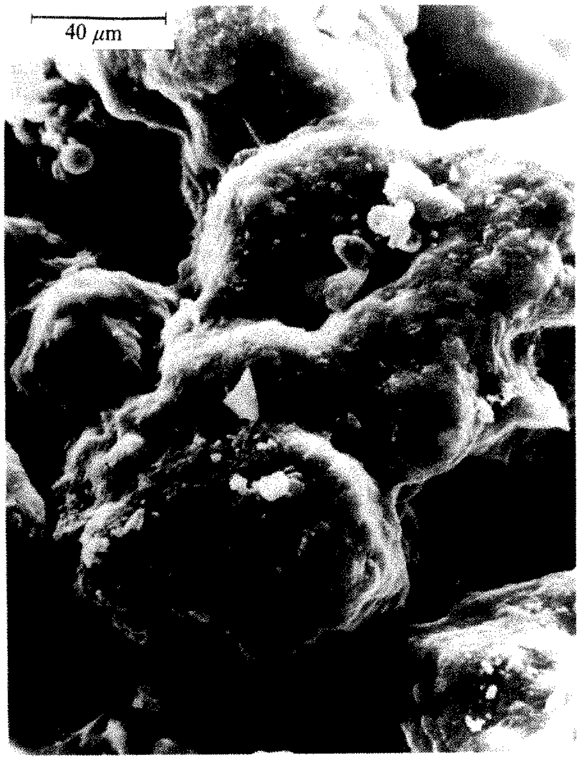

Fig. 2 Scanning electron micrograph of typical desert-varnish surface. Mound-like or botryoidal structures enlarge, coalesce, and are succeeded by new mounds as varnish accretes. Windblown-dust particles and microfloras visible on surface collect primarily in depressions, and become trapped in the varnish layer. Depressions may also shelter microorganisms. Cross-sections through a similar varnish surface are shown in Fig. 1.

IR spectroscopic measurements of chemical extracts that the main minerals are mixed-layer illite–montmorillonite clays with an intimate mixture of birnessite [(Na, Ca, K) $Mn_7O_{14} \cdot 3H_2O$] and hematite (Fe_2O_3). However, they found no X-ray powder-diffraction evidence for oxides. Our X-ray diffraction measurements also did not reveal definite peaks of oxides, and we agree with Potter and Rossman that any oxides present must be very fine grained (<0.1 μm). We found relatively strong peaks at ~12, ~10, ~4.50, ~3.36 and 2.57 Å, which may be caused by detrital clays, micas, feldspar quartz and so on, but which also are consistent with a possible Mn- and Fe-rich silicate mineral. We consider the present X-ray diffraction data ambiguous in veiw of the known heterogeneity of the scraped varnish samples.

Table 1 *a*, Electron microprobe analyses of varnish layers from Arizona*; *b*, total water§ in scraped Arizona varnish and in wind-deposited soil

a

Oxide	Basalt Substrate†	Band area 1 Black	Band area 1 Red	Band area 2 Black	Band area 2 Red	Band area 3 Black	Band area 3 Red
SiO_2	54.21	26.93	34.11	26.74	31.10	24.25	30.83
TiO_2	0.25	0.48	0.77	0.68	0.53	0.67	0.58
Al_2O_3	27.14	18.78	24.84	21.25	26.45	20.93	26.16
$Fe_2O_3^{(2)}$	2.08	17.41	23.54	18.44	24.51	13.45	24.83
$MnO_2^{(2)}$	0.00	20.46	2.92	17.52	2.47	24.41	2.94
MgO	0.14	2.44	2.24	1.92	3.42	1.70	2.91
CaO	9.36	0.98	0.50	1.11	0.76	1.25	0.87
Na_2O	4.28	0.24	0.22	0.23	0.35	0.22	0.20
K_2O	2.31	1.38	2.04	1.34	1.92	1.40	1.84
P_2O_5	0.13	1.08	1.18	1.43	1.38	1.34	1.14
S	0.03	0.14	0.23	0.16	0.15	0.19	0.18
Total	99.83	90.32	92.59	90.82	93.04	89.91	92.48

b

Desert varnish	9.19% + 0.28% H_2O (total)
Arizona soil	4.48% ± 0.13% H_2O (total)

* Sample from Fig. 1*a*, given in wt% oxide.

† Analysis is representative of feldspar-rich area below band areas 1–3.

‡ Fe computed as Fe_2O_3; Mn computed as MnO_2.

§ Analyses carried out on a DuPont moisture analyser by E. K. Gibson, Jr, at NASA Johnson Space Center. Samples were heated to 1,100 °C in a flowing stream of nitrogen. Released water was trapped on a P_2O_5 cell and removed by electrolytic conductance. Sensitive to 0.1 μg of water.

Thin sections show that the varnish layers consist of mineral plates orientated parallel to the layering. The plates range from submicron size to a few microns long, and have perfect basal cleavage. They are strongly pleochroic with α = golden yellow, $\beta = \gamma$ = deep reddish brown (~10-μm sections). Basal sections show $2V_\alpha \simeq 0°$. The refractive index in all orientations is >1.795. Microprobe analyses within large (2–3 μm) grains show them to be chemically homogeneous; however, the compositions of the grains vary from layer to layer, reflected in the analyses of Table 1. Thus, grains in dark layers are more strongly pleochroic and are more Mn-rich. We are not certain whether the grains are mixed phases (clays and oxides?) or whether they are a single silicate (unidentified) phase.

We interpret the layered botryoidal structures (Figs 1 and 2) as evidence that varnish grows vertically and laterally from nuclei in a manner similar to algal stromatolites. This conclusion is further supported by field evidence in Arizona where varnish is in all stages of re-covering spalls on boulders and outcrops of basalt. Old surfaces are completely covered with up to 200 μm of blue-black varnish, whereas surfaces of intermediate age show patchy coatings of lesser thickness. Field and laboratory observations indicate that freshly spalled surfaces are repopulated by microscopic black spots of varnish. Further addition of varnish occurs by enlargement of the spots which grow vertically and laterally. An analysis of one of these spots is given in Fig. 3. The spots progressively enlarge in size and number

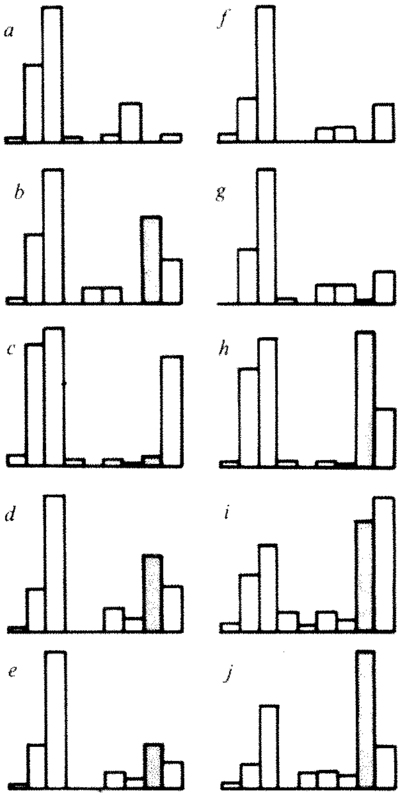

Fig. 3 SEM non-dispersive X-ray analyses (EDAX) of typical desert varnishes compared with aeolean dust, basalt substrate and a marine ferromanganese nodule. The red and black-band data* were approximated from Table 1. *a*, Arizona basalt substrate; *b*, varnish nucleation zone on Arizona basalt; *c*, Arizona varnish red band*; *d*, Idaho varnish; *e*, California varnish; *f*, Arizona soil near basalt; *g*, Arizona basalt surface outside varnish nucleation zone; *h*, Arizona varnish black band*; *i*, Utah varnish; *i*, marine manganese nodule.

until they coalesce and completely cover the substrate. Further thickening of the varnish occurs by continued nucleation and growth of spots (mounds) (Fig. 2). No evidence was found for the initial generation or growth of varnish as a uniform thin layer that simply increases in thickness with age.

The chemistry (Fig. 3), mineralogy and layered structure of varnish coatings from diverse localities and substrates are remarkably consistent, suggesting a common process of formation. We conclude from the lack of correlation between the composition of varnish and that of the substrate, and the presence of fine-grained dust on all sampled surfaces, that windblown dust is the source of the varnish materials. The preferential development of varnish on the tops of talus boulders is consistent with this view. This conclusion agrees with earlier suggestions[13] and with the results of Potter and Rossman[3,4].

Arizona varnish has concentrated manganese by 10^2–10^3 (Table 1, Fig. 3) relative to airborne dust that was collected in depressions on the tops of varnished talus boulders. If varnish is nourished by dust it implies not only an effective concentration mechanism, but also an efficient mechanism for the removal of large quantities of dust from which Mn and Fe have been extracted. The removal mechanism is probably rain and dew as is suggested by accumulations of soil in drip-evaporation deposits on the undersides of boulders that are perched above soil-level on talus piles. However, neither the mechanism for manganese (and iron) concentration nor the time-scale of the deposition is known. The repeated layering in the varnish implies that manganese deposition is favoured at certain times, followed by periods when less manganese is laid down. We propose that the cyclic concentration and deposition of MnO_2 are related to environmental changes, perhaps on a seasonal or annual basis, or on a longer time-scale separating periods when climatic conditions became optimum for varnish growth.

The highly effective mechanism for concentrating manganese, and the possibility of climatic control over the cyclic banding suggests (but does not prove) that a biological process may be involved in the formation of varnish[7,8,10,14]. The generation of varnish on rock by coalescence of growth centres is consistent with a biological process. We and J. Staley are studying the microorganisms that are associated with rock varnish. Cultures have been made in the field and in the laboratory of the surfaces and the interiors of varnishes. An assortment of bacteria, fungi and algae consistently occur on and within the varnish coatings, including at least one Bacillus that oxidises Mn^{2+} to Mn^{4+}. Further work is needed to determine whether organisms produce the varnish or merely live in it.

Knowledge of the processes responsible for the formation of desert varnish coatings may be useful in understanding present and ancient weathering environments, and in dating exposed surfaces[5,13,15]. The interpretation of optical remote-sensing data from satellites and aircraft must take into account the presence and significance of varnish in any attempt to map lithologies, as the coating locally masks the underlying rocks. If we can determine how varnish is deposited we may gain insight into the origin of remarkably similar layered deposits, namely freshwater and marine manganese nodules[16–21].

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Mantle $^{40}Ar/^{36}Ar$ trapped in Cretaceous deep-sea basalts

THE abundance and isotopic composition of rare gas in the mantle provides an important constraint on the origin and evolution of the Earth's atmosphere. One of sources of such information is basalts which erupted from ocean ridges. Ozima[1] stated that a high $^{40}Ar/^{36}Ar$ ratio in the mantle suggests sudden degassing at an early stage of the Earth's evolution. Several authors[2,3] have reported excess ^{40}Ar and high $^{40}Ar/^{36}Ar$ ratios in rapidly quenched rims of young deep-sea basalts. However, the Ar composition in old ridge basalts was not known. We report here a measurement of the isotopic composition of Ar in old deep-sea basalts. The Glomar Challenger drilled a Cretaceous ocean floor near the southern end of the Bermuda Rise in Deep Sea Drilling Project[4]. The drilled site (Site 417) is on the magnetic anomaly MO which has been estimated to be 108 Myr old.

The samples used were pillow basalts from Hole 417D, which contained only limited alteration minerals and had widespread fresh glass[4,5]. Glassy rims of the Cretaceous pillow basalt cores, designated 22-3/24-27, 28-5/85-92, and 29-6/95-104, were investigated for rare-gas composition. (Detailed results will be published elsewhere.) The concentration and isotopic ratio of Ar are given in Table 1. The potassium content in the basaltic

Table 1 Concentration and isotopic ratio of Ar in Cretaceous deep-sea basaltic glass

Sample	1	2	3	4	5	6
Core	22-3/24-27		28-5/85-92		29-6/95-104	
$^{36}Ar(10^{-9}\ cm^3\ g^{-1})$	1.44	2.14	1.65	4.42	0.998	5.95
$^{38}Ar/^{36}Ar$	0.194±0.002	0.187±0.003	0.191±0.002	0.186±0.002	0.188±0.004	0.190±0.004
$^{40}Ar/^{36}Ar$	1,341±7	819±22	1,103±7	557±7	1,686±11	567±17
$^{40}Ar^*/^{36}Ar$	1,090	650	861	466	1,353	511

* Corrected for radiogenic ^{40}Ar produced in basaltic glass for 108 Myr.

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glass has been determined to be 0.083, 0.091 and 0.075% for cores 22-3/24-27, 28-5/85-92 and 29-6/95-104, respectively (T. Ui, H. Haramura & H. Nagai, unpublished data). The radiogenic ^{40}Ar produced in basaltic glass during the past 108 Myr was corrected on the basis of the potassium content given above. The ^{40}Ar/^{36}Ar ratios corrected for radiogenic ^{40}Ar are also given in Table 1.

On the model that a given mantle component of Ar in the basalt mixed with varied amounts of contaminating atmospheric Ar, a plot of ^{40}Ar/^{36}Ar against 1/^{36}Ar gives a straight line,

$$({}^{40}\mathrm{Ar}^*/{}^{36}\mathrm{Ar})_{\mathrm{meas}} = S/({}^{36}\mathrm{Ar})_{\mathrm{meas}} + ({}^{40}\mathrm{Ar}/{}^{36}\mathrm{Ar})_{\mathrm{contam}} \qquad (1)$$

where ^{40}Ar*/^{36}Ar is the ratio corrected for radiogenic ^{40}Ar, and

$$S = ({}^{36}\mathrm{Ar})_{\mathrm{mantle}}[({}^{40}\mathrm{Ar}/{}^{36}\mathrm{Ar})_{\mathrm{mantle}} - ({}^{40}\mathrm{Ar}/{}^{36}\mathrm{Ar})_{\mathrm{contam}}] \qquad (2)$$

if the concentrations of mantle ^{36}Ar and ^{40}Ar are uniform in the samples investigated (see Fig. 1). To evaluate the amount of mantle ^{40}Ar and the ^{40}Ar/^{36}Ar ratio of mantle Ar trapped in the Cretaceous basaltic glass, the ^{40}Ar/^{36}Ar isotopic ratio for contaminating Ar and the concentration of mantle ^{36}Ar should be known.

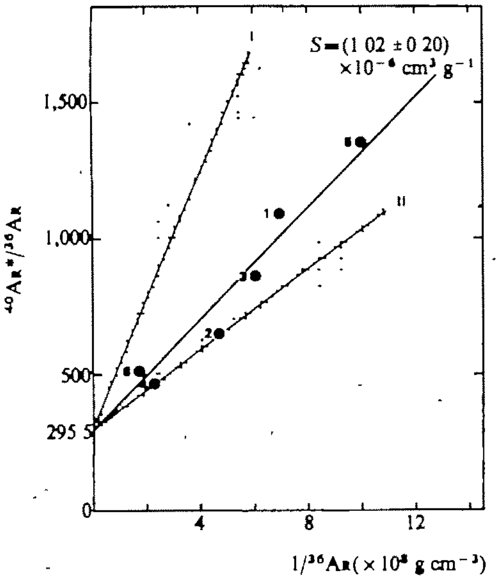

Fig. 1 A plot of ^{40}Ar*/^{36}Ar against 1/^{36}Ar. ^{40}Ar*/^{36}Ar ratios are corrected for radiogenic ^{40}Ar produced in basaltic glass by decay of ^{40}K for 108 Myr. The isotopic ratio of contaminating Ar was assumed to be atmospheric (see text). For comparison, correlation lines I and II given by Dymond and Hogan[8] are also plotted. ●, This work.

Microscopic examination of thin sections of the same cores used in this work (T. Ui, personal communication), shows that cores 22 and 29 have many cracks in the glassy rims along which alteration of glass is advanced. Gas pores have been filled with altered materials. For core 28, the alteration is so advanced that fresh glass is a minor component, and large cracks have been filled with altered materials such as carbonate, clay and zeolite. Thus the alteration in these cores is likely to be due to seawater–rock interaction at low temperature[5]. Hence the contamination of Ar can be attributed to penetration of seawater into the altered materials. Seawater which is in equilibrium with the atmosphere contains a large amount of atmospheric Ar (10^{-4} cm^3 STP g^{-1}) (ref. 6). Owing to a high diffusion rate of Ar in water, Ar in the altered materials could be replaced by Ar with the modern ^{40}Ar/^{36}Ar ratio.

Another possibility is contamination by seawater Ar at the time of eruption through the Ar diffusion into the quenching glassy rim of pillow basalt. In this case, $(^{40}\mathrm{Ar}/^{36}\mathrm{Ar})_{\mathrm{contam}}$ may be different from that of atmospheric Ar and represent the isotopic ratio of Ar in Cretaceous seawater. However, experimental evidence for ^{3}He and ^{40}Ar excesses in glassy rims of modern pillow basalts[2,3,7], indicates that the inherent rare gases in lava were compressed tightly in the rapidly quenched rims of pillow basalts under great hydrostatic pressure and the interaction with seawater was not appreciable in the rare-gas composition. Thus we presume that the contribution from the latter process is ineffective compared with the contamination of atmospheric Ar by the penetration of modern seawater into the altered materials. A least-squares fit gives an intercept of $(^{40}\mathrm{Ar}/^{36}\mathrm{Ar})_{\mathrm{contam}}$ equal to 243±73 without any assumption on the isotopic ratio of contaminating Ar. This value is consistent with that for atmospheric Ar (=295.5) within experimental error. In the following discussion, therefore, we use the atmospheric ^{40}Ar/^{36}Ar ratio as the isotopic composition of contaminating Ar.

Figure 1 plots ^{40}Ar/^{36}Ar against 1/^{36}Ar with the intercept of 295.5 for the Cretaceous deep-sea basalts. It defines a correlation line the slope of which is $(1.02 \pm 0.20) \times 10^{-6}$ cm^3 STP g^{-1}. For comparison, correlation lines for modern deep-sea basalts given by Dymond and Hogan[8] are also plotted. As mentioned earlier, the amount of mantle ^{40}Ar and the ^{40}Ar/^{36}Ar ratio of mantle Ar trapped in the Cretaceous basaltic glass depend on the concentration of mantle ^{36}Ar. We use the lowest concentration of ^{36}Ar measured in sample 5 as an upper limit of mantle ^{36}Ar. From equation (2) with this value, $(^{40}\mathrm{Ar}/^{36}\mathrm{Ar})_{\mathrm{contam}} = 295.5$, and $S = 1.02 \times 10^{-6}$ cm^3 STP g^{-1}, we have

$$1.02 \times 10^{-6} < ({}^{40}\mathrm{Ar})_{\mathrm{mantle}} \leq 1.31 \times 10^{-6}\ \mathrm{cm^3\ STP\ g^{-1}}$$

and

$$({}^{40}\mathrm{Ar}/{}^{36}\mathrm{Ar})_{\mathrm{mantle}} \geq 1{,}350$$

as a lower limit of the ^{40}Ar/^{36}Ar ratio of mantle Ar trapped in the Cretaceous deep-sea basalts. For mantle ^{36}Ar trapped in glass of modern deep-sea basalts, Craig and Lupton[9] have adopted 0.5×10^{-9} cm^3 STP g^{-1}. With this value, we have $(^{40}\mathrm{Ar})_{\mathrm{mantle}} = 1.17 \times 10^{-6}$ cm^3 STP g^{-1}, and $(^{40}\mathrm{Ar}/^{36}\mathrm{Ar})_{\mathrm{mantle}} = 2{,}340$ in the Cretaceous deep-sea basalt glass.

In conclusion, a linear correlation in the ^{40}Ar/^{36}Ar against 1/^{36}Ar plot indicates that Ar in the Cretaceous basaltic glass is a mixture between a uniform concentration of mantle Ar and varied amounts of contaminating atmospheric Ar. The ^{40}Ar/^{36}Ar ratio of mantle Ar trapped in the Cretaceous basaltic glass is >1,350, and the amount of mantle ^{40}Ar resembles that trapped in modern basaltic glass[10,11].

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Evolution of sulphur dioxide tolerance in perennial ryegrass

THE combustion of fossil fuels for domestic and industrial purposes, coupled with rapid industrial growth over the past 150 years, has resulted in sulphur dioxide becoming an integral part of the environment. Extensive research into the effects of sulphur dioxide on vegetation began in the 1920s, but the possibility of evolution of tolerance has only recently received attention, in spite of the implications of this for the overall effect of SO_2 in the field. We present here data that provide strong evidence for the evolution of SO_2 tolerance in a perennial ryegrass population from an urban area of Merseyside, UK.

Any environmental factor that has an adverse effect on plant growth may exert selection pressure on plant populations by acting on their inherent genetic variability, thus causing major changes in gene frequency within a few years[1]. Recent research has shown the detrimental effect of SO_2 on perennial ryegrass (*Lolium perenne* L.) and the existence of substantial genetic variability in response[2,3]. Such variation in response in the field could result in the elimination of susceptible individuals from natural ryegrass populations, thus causing an increase in the overall tolerance of these populations. Indeed, the indigenous ryegrass at Helmshore (24 km north of Manchester) seems to have evolved tolerance to sulphur dioxide. The observations at Helmshore, however, were based on the response of two ryegrass clones—selected as sulphur dioxide tolerant in field trials at Helmshore—compared with many individuals of the S23 cultivar grown from seed.

To investigate overall population responses and variability in genotype response to sulphur dioxide, we have examined two long-established perennial ryegrass populations from Merseyside. These have experienced widely different ambient sulphur dioxide concentrations[4], but the climate, soil and management conditions have been comparable, because they were collected from parklands similar in aspect, altitude and soil.

One population was collected from Newsham in the centre of Liverpool and the other from West Kirby, a rural area on the Wirral peninsula 16 km WSW of Liverpool. Records show that typical winter mean concentrations of SO_2 in the centre of Liverpool[4] between the late 1950s and the early 1960s were 600–700 $\mu g\ m^{-3}$. Since that time, the winter concentrations have decreased to approximately 200 $\mu g\ m^{-3}$. In rural areas of the Wirral however, concentrations have been much lower, decreasing from approximately 80 $\mu g\ m^{-3}$ in the early 1960s to 30–40 $\mu g\ m^{-3}$ at present.

Table 1 Response of two perennial ryegrass populations from Merseyside to sulphur dioxide

	Control ($35\ \mu g\ m^{-3}$)	Polluted ($650\ \mu g\ m^{-3}$)
(*a*) Living dry matter		
Newsham (high SO_2 area)	0.91	0.82
West Kirby (low SO_2 area)	0.90	0.64
$P < 0.05$ LSD between populations = 0.08		
LSD between treatments = 0.14		
(*b*) Total (living + dead) dry matter		
Newsham	1.18	1.18
West Kirby	1.19	1.00
$P < 0.05$ LSD between populations = 0.10		
LSD between treatments = 0.19		

Values are mean shoot dry weight (g per clone). Plants were grown under a 16 h day/8 h night cycle under a light intensity of 30 $W\ m^{-2}$. Day and night temperatures were maintained at 20 ±1°C, and 14 ±1°C respectively. Relative humidity was kept above 60%. Wind speed over the plants was 120 $m\ min^{-1}$. The results are the mean shoot dry weights of two sequential experimental runs with identical sets of clones carried out in the same fumigation conditions.

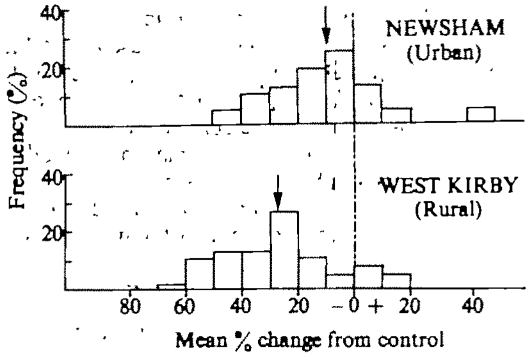

Fig. 1 Differential response to SO_2 of urban and rural ryegrass populations. Change from control measured as weight of living dry matter grown in SO_2 polluted air. Arrows mark arithmetic means of the frequency histograms.

Thirty-six individually identified clones of each population were exposed to 35 $\mu g\ SO_2\ m^{-3}$ (control) or 650 $\mu g\ m^{-3}$ (polluted) for 8 weeks in a wind-tunnel fumigation system (Table 1). In control air the mean yields of both populations were comparable. However, in the polluted air the West Kirby population showed a significant reduction in yield, whereas no significant reduction was shown by the Newsham population. Analyses of variance for both the living and total dry matter showed that there was a significant ($P < 0.01$) interaction between sulphur dioxide and the populations. Interestingly, the reduction in yield of total dry matter was less than that for living dry matter only. This suggests that the higher sulphur dioxide concentration caused an increase in the rate of senescence as well as 'invisible injury' (that is, reduction in yield without visible symptoms).

The percentage change in the living dry matter of each individual clone, as a result of the sulphur dioxide fumigation (Fig. 1), shows a wide range of genotypic variability within each population. In addition to the differential response of the populations to sulphur dioxide, the histograms reveal a reduced clonal variability within the Newsham population, presumably due to selection of the more tolerant genotypes by sulphur dioxide in the field.

These results provide strong evidence that the high sulphur dioxide concentrations prevalent in urban areas of Merseyside until the early 1960s have acted as a selective force. Furthermore, this population differentiation has persisted even though sulphur dioxide concentrations have decreased appreciably since then.

It is, therefore, important that the evolution of sulphur dioxide tolerance is taken into account when attempts are made to estimate reductions of yield in the field by extrapolation from laboratory studies. Fumigation of spaced plants in laboratory experiments may well overestimate the effects of sulphur dioxide on plant populations, as there can be no compensation for the yield reduction of sensitive individuals by the increased growth of tolerant individuals in these experiments, as would occur in dense swards. If the evolution of tolerance were sufficiently rapid in young grass swards then overall productivity would suffer only a temporary reduction, provided that the more tolerant individuals were of comparable vigour to the more susceptible. This seems likely, for in our results the yields of the two populations were similar in control treatments. Unfortunately, little is known about the rate of evolution of sulphur dioxide tolerance, although it is known that evolution of metal tolerance can occur within a few years.

In the field, plants are often exposed to mixtures of air pollutants, and the yields of some pasture grasses can be reduced severely by the synergistic interaction of sulphur dioxide and nitrogen dioxide[5]. It is thus possible that some plant populations could have evolved tolerance to combinations of air pollutants as well as to a single pollutant.

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The potential for evolutionary adaptation to sulphur dioxide depends on the existence of genetically based variability in response within the population, variety or species. Such variability would be less in inbred agricultural crop varieties: also it is unlikely that substantial genetic change would occur in any crop, however variable, that was replaced annually from material bred in a low sulphur dioxide area.

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Rhizobium japonicum mutants unable to use hydrogen

FACTORS which limit nitrogen fixation by the legume nodule symbiosis may be environmental (such as illumination, water or CO_2 supply), or intrinsic (such as supply of photosynthate to the nodule[1], effectiveness of rhizobial strain). An important intrinsic feature seems to be ATP-dependent hydrogen evolution by nitrogenase, which leads to loss of energy that would otherwise be available for nitrogen fixation[2]. Some strains of *Rhizobium japonicum* possess a unidirectional hydrogenase, capable of hydrogen oxidation but not hydrogen evolution, which recycles the hydrogen evolved by nitrogenase, regenerating ATP as well as protecting the nitrogenase from oxidative damage[3–5]. The physiological importance of hydrogenase in this context, with its obvious implications for agriculture, has so far been deduced from studies on naturally occurring strains possessing or lacking hydrogenase[2,6]. A more accurate assessment requires the isolation of isogeneic hydrogen uptake-negative (Hup^-) mutants from hydrogenase-positive (Hup^+) parents, and their comparison in physiological and agronomic studies. Such mutants would also be valuable for gene transfer and biochemical studies. We now report the isolation of Hup^- mutants.

Table 1 Effect of phenazine methosulphate on viability of bacteroids

Conditions	No. of survivors	% Kill
No PMS, N_2 atmosphere	4.2×10^9	—
PMS, N_2 atmosphere	2.8×10^9	23%
PMS, H_2 atmosphere	6.0×10^8	86%

A bacteroid suspension of strain USDA DES 122 from fresh soybean nodules was divided among test tubes sealed with serum stoppers and then flushed with the appropriate gas. Phenazine methosulphate (1 mM) was added, and the suspension incubated at 26 °C in the dark. After reduction of the dye (5 min), the atmosphere was replaced with O_2 and the suspension was rapidly mixed under normal laboratory illumination Cells were centrifuged, resuspended and plated for survivor counts. The number of survivors reported is the average of two separate experiments. The bacteroid suspensions consisted of 2.0 mg dry weight bacteroids in 0.4 ml of Mg-phosphate buffer (50 mM K_2HPO_4, 2.5 mM $MgCl_2$, pH 7.0) and were prepared as described elsewhere[15], except that the bacteroids were washed twice in the Mg-phosphate buffer

Our strategy was to seek a substrate for hydrogenase whose product on reduction was, or could be made, toxic. Survivors resistant to such toxicity ought to include Hup^- phenotypes. 'Phenazine methosulphate' (N-methylphenazinium methyl sulphate, PMS) proved to be a good substrate for rhizobial hydrogenase[4] and was reduced faster by bacteroid suspensions in the presence of H_2 than in its absence. In pilot experiments with *Azotobacter vinelandii*, photo-oxidation of PMS reduced in H_2 by bacteria killed some 90% of the cells; we therefore devised the procedure in Table 1, in which suspensions of largely viable[7] bacteroids were allowed to reduce PMS and immediately treated with pure O_2; enhanced killing in the presence of H_2 was then observed. The reason for the lethal effect of oxidising reduced PMS is not clear; it may involve H_2O_2 formation[8], a damaging effect of a free-radical derivative of PMS[9] or localised generation of the antibiotic pyocyanine from PMS.

Survivors of the PMS-treated population were then screened by the triphenyl tetrazolium procedure reported from this laboratory[10]. To be confident that presumptive mutants were not random contaminants, it was necessary to label genetically the strain treated, and so a parent strain (SR) was prepared: a spontaneous streptomycin (Str, 250 μg ml^{-1})-resistant followed by kanamycin (Kan 50 μg ml^{-1})-resistant double mutant of strain USDA DES 122. This strain showed effectiveness, hydrogenase content and nodulation capacity comparable to DES 122 and retained the Kan and Str markers when re-isolated from nodules.

Table 2 H_2 evolution, C_2H_2 reduction, nodule weight and number of nodules formed by strain SR and derivative mutants of *R. japonicum*

Strain	H_2 evolution (μmol per h per g nodule wt)	C_2H_4 formed (μmol per h per g nodule wt)	Nodule weight (mg per plant)	No. of nodules per plant
SR	<0.005	73.9±2.3	193±14	27±2
SR1	24.8±2.4	54.6±3.4	162±19	23±5
SR2	18 5±1.9	46.9±2.3	243±22	27±3
SR3	24.0±2.9	56.4±7.0	217±18	22±3

Results are means ±s.e.m. for three 25-d-old Wilkin soybeans grown in Leonard jars in the greenhouse, as described elsewhere[11,12]. At the time of planting, each seedling was inoculated with approximately 1×10^9 cells. Nodulated roots were placed into 30-ml serum vials, the vials were sealed and incubated for 15 min at 26 °C, and 0.5-ml samples removed for H_2 quantitation by gas chromatography. After removal of the H_2 sample, 3 ml of C_2H_2 was injected into the vial, and 0.5-ml gas samples were removed at 15 min and 30 min for C_2H_4 quantification by gas chromatography.

For PMS treatment, nodules produced by this strain were picked from 4-week-old soybean plants (cv. Wilkin) grown in Leonard jars[11,12], and bacteroids were obtained as described in the legend to Table 1. The conditions of PMS treatment were also as described in the Table 1 legend, except that the suspensions contained 2.4 mg bacteroids. Survivors of the PMS killing procedure were plated on to a medium previously described[13], but also containing gluconate 5 g l^{-1}, arabinose 5 g l^{-1}, triphenyl tetrazolium chloride 0.1 g l^{-1} and streptomycin 250 μg ml^{-1}. Approximately 1.7×10^4 colonies formed by individual survivors of the PMS treatment were screened for tetrazolium reduction. Those colonies exhibiting low dye-reduction ability after 10 d of growth were chosen and restreaked on to the tetrazolium-containing medium. The isolates consistently showing poor dye-reduction ability were each inoculated on to two soybean plants in cellophan growth pouches[14]. After 3 weeks of growth the roots from each pouch

Table 3 H_2 and O_2 uptake by bacteroids and free-living cultures of strain SR and mutant strains SR1, SR2, SR3

Strain	Bacteroids		Free-living cells	
	H_2 uptake*	O_2 uptake*	H_2 uptake†	O_2 uptake†
SR	3.7	0.70	119	230
SR1	<0.005	0.64	<0.005	276
SR2	<0.005	0.71	<0.005	240
SR3	<0.005	0.82	<0 005	253

* Expressed as μmol per h per mg protein. Nodules from 28-d-old Wilkin soybean plants were collected and the bacteroid fraction isolated in aerobic conditions as described in the legend to Table 1. Bacteroid suspensions containing approximately 1.5 mg protein per ml in Mg-phosphate buffer were assayed for uptake of H_2 and O_2 at initially saturating levels[11] by use of an amperometric method that used a Clark-type electrode[16]. Rates of O_2 uptake were determined in the absence of H_2.

† Expressed as nmol per h per 10^8 cells. Cells were grown for 5 d on a medium designed for expression of hydrogenase[10], suspended in Mg-phosphate buffer, and O_2 and H_2 uptake rates determined amperometrically. All suspensions contained $1.1–1.2 \times 10^8$ cells per ml. Rates of O_2 uptake were determined in the absence of H_2.

were removed from the plants, and the nodulated root system was assayed for H_2 evolution by gas chromatography. Nodules lacking hydrogenase are not able to recycle H_2 produced by nitrogenase, so Hup^- mutants would evolve H_2.

Three mutants which produced nodules evolving H_2 were obtained, SR1, SR2 and SR3; strain SR evolved no H_2 (Table 2). The three mutants did not differ greatly from the wild type in symbiotic characteristics other than the ability to use H_2 (Table 2). Nodule weight and number of nodules formed per plant for SR2 and SR3, were not appreciably different from the corresponding values for SR. Apparently, their lack of ability to use H_2 did not greatly impair their other symbiotic properties. Mutant SR1, however, showed less nodule weight than SR, SR2 and SR3. Strain SR apparently formed nodules containing greater nitrogenase activity than any of the mutants (Table 2, and other data not shown); this greater nitrogenase activity may be due to the ability of strain SR to recycle H_2. The Hup^- phenotype of the mutants was retained on re-isolation from nodules, together with the Kan and Str markers; hence, the Hup^- mutations were reasonably stable. Free-living cultures were grown in the conditions previously reported[10] which allow expression of Hup^+ *ex planta*, and were tested for H_2 and O_2 uptake rates by the amperometric method[11,16]. Bacteroid suspensions were also tested. Suspensions of strain SR took up H_2 but none of the mutants did so (Table 3). Free-living cultures of the mutants as well as bacteroids isolated from nodules showed O_2 uptake rates comparable to that of strain SR (Table 3), indicating that the mutant strains still carry out normal O_2-linked respiration.

Three mutants obtained by screening 1.7×10^4 survivors of the PMS treatment is rather a high proportion, so the possibility exists that the PMS procedure is mutagenic. We found that the PMS procedure approximately doubled the frequency of streptomycin-resistant isolates (from bacteroids of *R. japonicum* strain USDA DES 122) compared to untreated controls; some possibility therefore remains that the mutants contain additional lesions in loci other than in the H_2-uptake system. It has not so far been practicable to check by conventional reversion tests whether the Hup^- strains are point mutations.

Greenhouse and field tests of these mutants are in progress to assess the agronomical importance of hydrogenase, and their specific biochemical defects will be investigated to aid understanding of the biochemistry and physiology of the H_2-uptake system in the *Rhizobium*-legume symbiosis.

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Kinship asymmetry in diploids

THE relatedness of one individual to another is an important variable in kin selection theory. An unusual property of relatedness is its asymmetry. For haplo-diploids, this asymmetry has been discussed[1]. We show here that, surprisingly, there may also be asymmetry in relatedness between two diploids. One may in fact be more related to a person than they are to you.

The easiest way to see this is to consider a single locus, where the genotypes of two individuals are known. Possible pairs of genotypes that the individuals may have are shown in Table 1. When one individual is homozygous, and the other heterozygous with one of its alleles common to both individuals, relatedness is not reciprocal. An individual A with genotype *ww* is related to an individual B with genotype *wx* by a half, but B is related to A by one. Note that B can increase the representation of its alleles more in the next generation by aiding A than A can by aiding B.

This asymmetry in relatedness of diploids follows from the concept of relatedness as applied to cases of inbreeding[2]. A

Table 1 Relatedness of A to B and B to A

Genotypes		Relatedness	
A	B	A to B	B to A
ww	*ww*	1	1
ww	*wx*	½	1
wx	*ww*	1	½
wx	*wx*	1	1
wx	*wy*	½	½
wx	*yz*	0	0
wx	*yy*	0	0
ww	*yz*	0	0
ww	*yy*	0	0

statement of relatedness, valid for diploids and haplodiploids alike is that the relatedness of A to B is a ratio of averages: the average, over alleles in A, of the probabilities that each will be found in a random gamete of B, divided by the average, over alleles in A, that each will be found in a random gamete of A.

In the usual kin selection analysis, one does not deal with the certainty of known genotypes, but rather with the overall expected relatedness (for example, expected frequencies of genotype pairs) that two individuals may have as the result of some pedigree relationship. Consideration of expected relatedness for diploids also gives non-reciprocal relatedness whenever there is some inbreeding and the two individuals are not identically inbred[2,10].

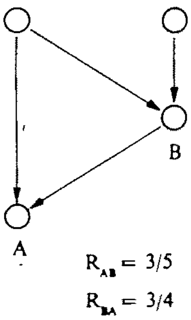

Fig. 1 Parent–offspring mating and relatedness

As it is unlikely that mating in natural populations is uniform and symmetric, non-reciprocal relatedness will be common. Simple pedigrees that have the property of non-reciprocal relatedness are brother–sister mating followed by one of the sibs outbreeding, or parent–offspring mating. A pedigree diagram for the latter case is shown in Fig. 1. Relatedness values are given as calculated from Hamilton's expression[2]. Both of these simple pedigrees occur in natural populations; for example, they are thought likely in wolves[3].

There is more asymmetry in relatedness if there is more inbreeding before the outbreeding. At the extreme limit, when inbreeding has reached essential homozygosity, offspring produced by outbreeding would be related to their inbred half-sibs by one; relatedness of the inbred individuals to their outbred half-sibs would be a half.

Although there is substantial literature on problems of calculating relatedness[2,4,5], non-reciprocal relatedness of diploids has escaped notice. Perhaps this is because the idea conflicts with everyday usage of the word.

Kin selection theory uses relatedness in proposing answers to two sorts of questions. One question concerns how an optimal strategist should act when faced with a choice of aiding or not aiding other individuals. The other question concerns the expected change in allelic frequency of an allele that has indirect effects on the fitness of individuals other than the allele's bearer. Non-reciprocal relatedness affects the answers to both questions but the implications for optimal strategy decisions seem the most interesting.

For haplo–diploid organisms, asymmetrical relatedness has recently been in the centre of attempts to test strategy aspects of kin selection theory[6–8]. In the diploid–diploid case, recognition of non-reciprocal relatedness might lead to testable predictions about interactions between individuals.

A major unresolved aspect of kin selection theory is the degree to which kin selection has actually resulted in effects on the evolution of phenotypes. Many aspects of behaviour and social structure have been interpreted under the kin selection paradigm. However, the theory is rather flexible, and it is easy to bend it to fit the sparse data available.

Perhaps the consequences of non-reciprocal relatedness are trivial and the magnitude or predictability of this asymmetry falls below the as yet unknown threshold for the evolution of strategies to take it into account. It is not yet clear what magnitudes of relatedness can influence the evolution of phenotypes. Empirical studies will be necessary to resolve this. Successful empirical testing requires awareness of the subtleties of relatedness if it is to succeed in establishing the domain of validity of kin selection theory. Pending such tests, I agree with Williams[9] that adaptation, particularly such 'fine-tuned' adaptation should not be assumed. The burden of proof lies with those who claim the adaptive significance of this or any phenomenon.

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Communal breeding in green woodhoopoes as a case for reciprocity

RECIPROCITY[1], or reciprocal altruism[2], and kin selection[3] are concepts basic to sociobiology. Although both theories have been widely accepted[4], little critical evidence exists for either[5,6]. Communal, or cooperatively breeding, birds are important organisms for testing these theories because nonbreeding helpers regularly provide aid to nestlings, such as food and protection. Helpers are often related to the nestlings they aid, frequently being older siblings[7,8], leading some authors to conclude that kin selection is the evolutionary basis for the apparent altruism seen in cooperative breeders[8,9]. However, the possible influence of other, individual strategies has not been completely separated from kin selection for any communal bird; this would require elimination of kinship ties as a variable[5]. Aid by a helper to an unrelated young bird must either be considered an 'accident' or it suggests that factors other than, or in addition to, kin selection might be the evolutionary bases for helping behaviour, at least in that particular species. Here we report information on a tropical, communal bird, the green woodhoopoe (*Phoeniculus purpureus*), that suggests helping is a strategy for personal gain[10].

We have studied the social behaviour of green woodhoopoes near Naivasha, Kenya, from July 1975 to the present. During the first period of field work (July 1975–May 1976), we individually marked almost all members of 24 flocks, some 148 birds. Subsequently, we have marked an additional 109 woodhoopoes, most of which were juveniles produced in these flocks. We have also recorded and marked immigrants into, and emigrants from, the original groups. Agonistic behaviour is common enough to permit us to assign dominance and subordinate status to most flock members. Thus, kinship ties and social statuses of many flock members are now known.

Social units or flocks of green woodhoopoes consist of 2–16 birds and contain a single breeding pair. Other flock members, usually relatives of one or both breeders, serve as nest helpers. When a flock is decimated, or when a new flock forms, 1–4 females merge with 1 or 2 males; sometimes these social units consist of individuals unrelated to either potential breeder. Unrelated birds of the same sex were usually females[11].

Woodhoopoes of all ages, sex and social classes disappear at a rate of about 40% per year (January 1976–January 1978); we equate most disappearances with mortality[12]. Because of this high mortality, we have obtained information in only three instances concerning the roles of unrelated birds in subsequent breeding activities.

First, in early June 1977 the L-h flock consisted of a single adult male, GL, banded in this territory in late 1975, and four females not present the previous January. Two females, GR and WR, were banded together as immatures in an adjacent territory in August 1975, and were almost certainly siblings. Two younger females, OL and RL, from outside the study area and thus not closely related to GR and WR, were also present. Male GL and female GR bred in early June, with the other three females behaving as nest helpers. RL and OL, unrelated to the nestling, provided more food than did the sibling (WR) of the female breeder (Table 1). Thus, differences in quantity of food delivered to this nest were apparently unrelated to previous foraging experience[13].

Food deliveries by RL and OL increased conspicuously as the nestling matured (Fig. 1). (The increased activity of these helpers was probably not due to increased needs of the single nestling as it grew. During the same period an unaided pair successfully reared two healthy young.) These two birds apparently sought to interact directly with the nestling rather than to deliver food to the female parent, GR. By 13 July, GR remained outside the nest cavity for extended periods, providing the opportunity for helpers RL and OL to enter the nest and feed the nestling directly; from then on their contributions increased proportionately more than did those of the male parent. By 24 July, the nestling perched at the cavity entrance and these helpers groomed and vocalised to the young bird, in addition to feeding it.

Second, in June 1977, the H5 flock consisted of an adult male, OL, his younger male sibling, WR, the adult breeding female, YL, banded in this territory in 1975, a juvenile female offspring, GL, of YL and her previous mate, and a new subadult immigrant female, WL, unrelated to the others. YL soon disappeared and WR returned to his previous territory. Male OL and female WL began courtship feeding and copulation. WL now also occasionally fed GL, then almost a year old and long self-sufficient, and initiated vocal rallies with her. Vocal rallies are an important intra-group behaviour promoting cohesion. In short, WL seemed to develop a closer personal bond with GL. When WL nested in early July, GL became an active nest helper (Table 1), although she was unrelated to either breeder. Female WL, a newcomer to the H5 territory and unrelated to the original breeding pair, thus inherited ownership of a territory, breeding status and a nest helper, GL, within a year or less of her arrival.

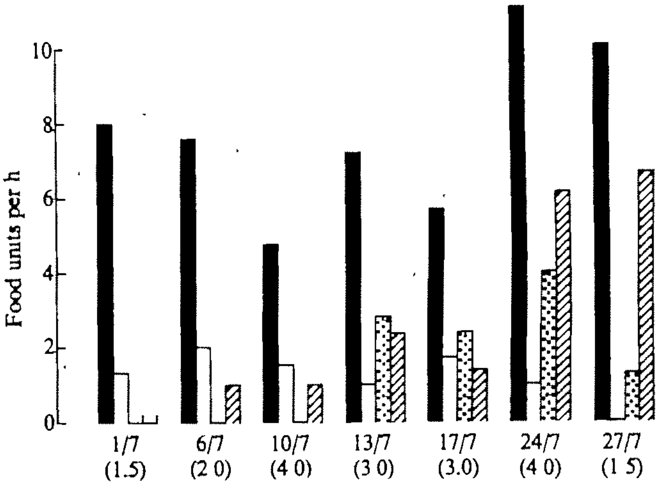

Fig. 1 Food contributions of _ flock members after the single nestling had hatched. Food units are based on size categories; large, medium and small, multiplied by feeding visits. Numbers on the horizontal axis indicate date and in parentheses, the number of hours observation on each date. Flock members are: ■, GL, male parent, □, WR, female sibling of female parent; ⛆, RL, and ▨, OL, females unrelated to either parent OL was not more than 1 yr old. On 13 July the female parent first remained outside the nest for extended periods. From this date on, contributions of RL and OL, in particular, increased proportionately more than did those of the male parent.

Third, in early September 1975, the AD flock consisted of an incubating female and two males. A single young hatched on 17 September. Two females (GR and YR) from an adjacent flock occasionally foraged with the males in the latter's territory as the female breeder incubated, but usually they remained in their own flock, where they helped feed recently fledged juveniles. These females did not visit the AD nest until after the female parent had disappeared, between 7 and 13 October. By the latter date both had joined the AD males, with GR assuming the role of foster mother.

The apparently conflicting interests of the four adults at this nest led to a great variation in feeding patterns. Female GR behaved in typical maternal fashion, often remaining in or near the nest as other flock members foraged elsewhere. When a bird approached the nest with food, GR quickly entered the nest to intercept the food item, presumably passing it on to the nestling.

Table 1 Activities of helpers at two green woodhoopoe nests in 1977

Flock: helpers	Stage of nesting cycle						
	Incubation			Nestling			
	Food units	Feding visits	Non-feeding visits	Food units	Feeding visits	Non-feeding visits	Direct interaction with nestlings*
L-h: (17)				(19)			
♂ GL†	96	45	0	144	71	3	Ca. 12‖¶
♀ WR	7	4	17	24	13	15	5¶
♀ RL	10	6	3	33	18	4	9¶
♀ OL	2	2	5	52	30	1	9¶
H5: (13)				(16)			
♂ OL†	102	54	2	159	89	1	1
♀ GL	32	16	7	114‡	64	6	8
♂ WR	0	0	0	33§	15	0	2

Food units are based on size categories of food brought to the nest: large (3), medium (2), small (1) × feeding visits. Numbers in parentheses indicate hours of observation.

* Includes entering the nest, vocalising directly into the nest, or grooming the nestling at the cavity entrance.

† Male breeders provided significantly more food both to the incubating female and to the nestling(s) than did the helpers (Mann–Whitney U).

‡ GL brought significantly more food to the nest then did WR (Mann–Whitney $U = 21$); GL is unrelated to the nestlings, whereas WR is an uncle.

§ Male WR returned to this territory at about the time the eggs hatched and subsequently fed the young.

‖ Male began to feed young directly when it came to nest entrance. Would not pass food to females other than GR, although several females attempted to take it.

¶ After 10 July both feeding and other interactions increased for the L-h helpers, when the female parent remained outside the nest for extended periods (see Fig. 1).

Female YR almost always gave food to GR, apparently to avoid aggression by the latter. Helper male WL strongly preferred to feed the nestling directly, but GR frequently thwarted him. For example, during a 4-h watch on 16 October, WL attempted 10 times to go directly to the nestling, but was intercepted each time by GR. The male breeder, GL, also frequently attempted to feed the nestling directly, but was equally likely to pass the food to his new mate, GR. GR seemed to behave in a manner designed to condition the males to accept her as the new breeder and to maximise her bonds with the unrelated nestling. The nestling fledged on 22 October and was cared for by all four adults until its disappearance between 13 and 18 November.

Our observations of 13 other flocks, where helpers were either related to the nestlings or where relationships were unknown, also support the suggestion that helpers vie for the opportunity to feed young. We have recorded 69 instances in which one helper has either stolen or begged food from another and then delivered it to the nestlings, and 38 cases where a helper actively avoided the soliciting female parent, to feed the young directly. These apparently disorganised behaviours at the nest make sense if helpers seek to establish personal ties with nestlings, as we suggest.

The data presented here suggest that nestlings, related or not, are valuable resources and that potentially it is to a woodhoopoe's advantage to feed and interact with them (helpers preen and vocalise vigorously to older nestlings), because the helper's subsequent attainment of breeding status and breeding success often depends on the presence of subordinate flock mates (our unpublished work); that is, nestlings are a potential resource for helpers.

In 1978 the contributions of female helpers GL and OL of the H5 and L-h flocks, respectively, were greater than in 1977, even though food was probably less plentiful. This second year of data strengthens the conclusion that such behaviour is adaptive, rather than aberrant.

The cases described here do not support an interpretation of helping behaviour relying on kin-selected altruism. The L-h and H5 flocks each contained a helper closely related to one of the breeders, as well as one or two helpers not related to either. At both nests, young received more food and other attention from unrelated helpers than from relatives. Thus, although most woodhoopoe flocks consist of relatives, these cases suggest that kinship ties (beyond parent–young) and helping behaviour in green woodhoopoes may not be causally related; instead, individual woodhoopoes can apparently gain by helping any young, at least in part because those young might later enhance the helper's future reproductive success.

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Effect of agrocin 84 on attachment of *Agrobacterium tumefaciens* to cultured tobacco cells

CROWN GALL DISEASE of dicotyledenous plants has aroused considerable interest, partly because of its economic consequences, and partly because it is the only known example of a bacterially induced cancer. The causative organisms are various strains of *Agrobacterium tumefaciens* harbouring large plasmids[1–3]. Parts of these plasmids are transmitted to plant host cells, where they are maintained and transcribed[4,5]. In horticultural conditions, biological control of the disease can be achieved by inoculating susceptible plants with a non-pathogen, *A. tumefaciens* K84 (refs 6, 7) and is due to the production of an intraspecific antibiotic, agrocin 84, by this strain[8]. Although the structure of agrocin 84 has been partially elucidated[9], the mechanism by which it prevents induction of crown gall is poorly understood. We report here the transformation of cultured tobacco cells to crown gall tumour cells and present evidence, using this *in vitro* transformation system, that agrocin 84 blocks the initial step of tumour induction, that is, the attachment of pathogen to host.

Table 1 Effect of agrocin 84 on *in vitro* transformation of habituated tobacco cells by *A. tumefaciens*

Strain	Nopaline content of extracts: Callus tissue (% dry wt)	Nopaline content of extracts: Cell medium (μg ml^{-1})	Tumour development on tobacco plants
C58	0.35	<0.04	+
C58 (agrocin-treated)	<0.01	<0.04	−
C58 (NT-1)	<0.01	<0.04	−
C58(NT-1) (agrocin-treated)	<0.01	<0.04	−

A. tumefaciens C58 and C58 (NT-1) were incubated for 12 h in peptone broth (0.4% Bacto peptone, 0.05% $MgSO_4$, 2% sucrose) and the logarithmically growing cells were collected by centrifugation at 5,000*g* for 15 min at 25 °C. After resuspension in MM growth medium[23] both strains were incubated in the presence or absence of purified agrocin 84 (unpublished data) for 2 h (12 unit ml^{-1}, where 1 unit ml^{-1} is the quantity required to reduce the first order growth rate constant by 50% under defined growth conditions). Aliquots of the four bacterial suspensions were inoculated onto wounded tobacco plants, embedded for electron microscopy and introduced into cultures of habituated tobacco cells maintained in Filner growth medium[24] and incubated at 25 °C in an orbital shaker. These tobacco cultures were incubated for 4 weeks, rifampicin (15 μg ml^{-1}) being added 48 h after infection. Plant cells were separated from growth medium and both were extracted for amino acids essentially as described by Bomhoff[25]. Guanido compounds were detected and estimated by modified versions of the Sakaguchi method[26,27]. A component of the aqueous extract from callus tissue infected with *A. tumefaciens* C58 was identified as nopaline by electrophoresis at *p*H 3.5 and paper chromatography[28] using authentic samples of arginine, octopine and nopaline as references.

A. tumefaciens C58 is a member of the large group of pathogens harbouring the nopaline-type tumorigenic (Ti) plasmids. In addition to coding for enzymes catalysing nopaline synthesis in transformed plant cells and nopaline hydrolysis in the bacteria, these plasmids code for components which render the bacteria sensitive to agrocin 84[2,8,10–13]. When strain C58 is exposed to agrocin 84, a few bacteria develop resistance to the antibiotic. They are avirulent[8] and resemble the 'heat-cured' derivative of strain C58 (named C58(NT-1)), in that they do not carry a Ti plasmid[2].

Many authors have suggested that the first step in the tumour induction process involves a specific interaction of a surface component of the bacterial envelope with wound-exposed

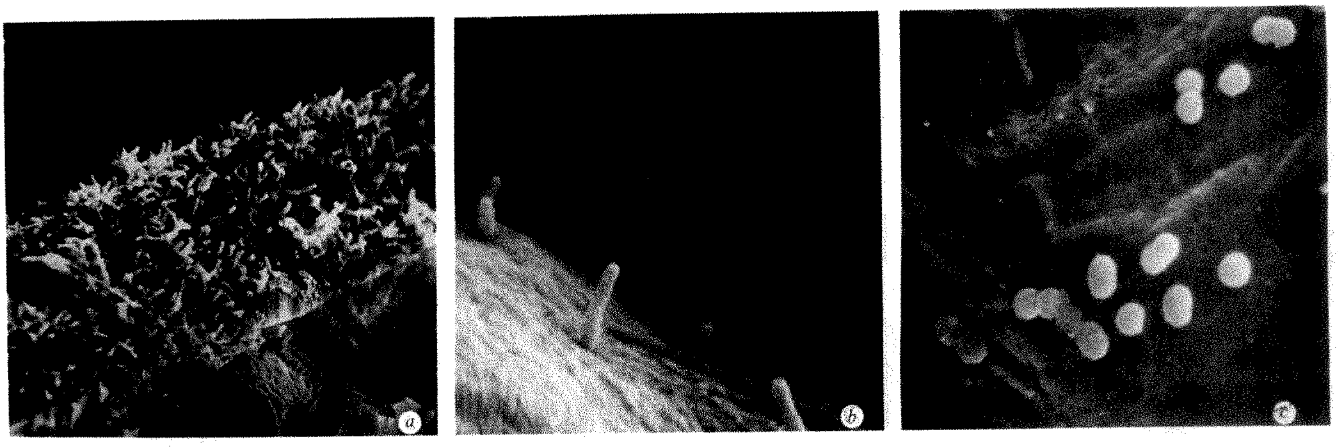

Fig. 1 Scanning electron micrographs of *A. tumefaciens* C58 attached to the surface of habituated tobacco callus cells, 4 h after infection. Magnification of *a*, *b* and *c*, ×1,740, ×34,000 and ×35,800 respectively. In *c* the pathogenic bacteria were preincubated with agrocin 84 for 2 h before inoculation into the tobacco cultures.

portions of the host cell wall[14–16]. Moreover, the correlation between pathogenicity and agrocin 84 sensitivity in *A. tumefaciens* C58 and other nopaline-utilising strains has led to the suggestion that surface receptors which facilitate the adherence of these bacteria to the host cell may also bind agrocin 84 (refs 13, 17). The early events of tumour induction are difficult to study using wounded plant tissue. Thus, by using *A. tumefaciens* C58 and its heat-cured, plasmid-free derivative, strain C58 (NT-1), to infect suspension cultures of habituated tobacco cells, we have developed an *in vitro* transformation system, amenable to a detailed investigation of the initial stages of tumorigenesis and of the role of agrocin 84 in preventing tumorigenesis.

The results of preliminary transformation experiments reported in Table 1 clearly indicate that the *in vivo* and *in vitro* transformation mechanisms are similar. The cultures of strain C58 which induced tumour formation on wounded tobacco plants also induced nopaline synthesis in habituated tobacco cells and both processes were blocked by the action of agrocin 84. Cultures of the heat-cured derivative, strain C58(NT-1), were not able to induce either *in vivo* or *in vitro* transformation. The concentration of nopaline found in C58 transformed tobacco callus cells was similar to that found in excised tumour tissue (J. Firmin, personal communication) and within the limits of detection (0.04 μg ml^{-1}), nopaline was not found in the incubation medium. This finding is perhaps significant in relation to the mechanism of transfer of tumorigenic DNA to non-pathogenic recipients: although octopine is known to increase the frequency of plasmid transfer in octopine-utilising strains of *A. tumefaciens*, nopaline does not enhance the *in vivo* frequency of plasmid transfer in a number of nopaline-utilising strains, including strain C58 (ref. 18).

The idea that *A. tumefaciens* attaches to specific sites in wounded plant tissue originated from the observation that tumour formation could be inhibited by the inclusion of avirulent strains of *A. tumefaciens* in inoculums of virulent strains[19]. If this is the case, then pathogenic and non-pathogenic strains would possess similar affinities for these sites. On the other hand, if an agrocin 84 receptor participates in the attachment of pathogen to host, then a marked difference in the binding properties of pathogenic and non-pathogenic strains would be expected. The *in vitro* transformation system permitted an investigation of these possibilities.

The infection experiments presented in Table 2 were carried out in conditions similar to those used for transformation, but in order to quantify the percentage of free to bound bacteria after a short infection period (4 h) strains C58 and C58(NT-1) were radiolabelled with ^{32}P before agrocin 84 treatment and the subsequent addition of the bacteria to suspension cultures of the plant host. The results obtained do not agree with the hypothesis that pathogenic and non-pathogenic strains have equal affinity for the host, but suggest that the surface properties of pathogenic and non-pathogenic strains of *A. tumefaciens* are different. Whereas over 90% of the pathogenic C58 strain remained associated with the tobacco cells after fractionation, only 15% of the non-pathogenic derivative C58(NT-1) was located there. Furthermore, pretreatment with agrocin 84 consistently reduced the affinity of strain C58 for its host by a factor of two to three, but did not significantly alter the binding capacity of strain C58 (NT-1). The observations made when the infected tobacco cells were examined under the scanning electron microscope corroborated our interpretation of the results of the binding experiments reported above and further, dramatically demonstrated that the formation of a specific infection complex is essential for tumorigenesis. This cytological study revealed that the rod-shaped, non-pathogenic bacteria of strain C58(NT-1) lay recumbent on the surfaces of the tobacco cells, randomly and sparsely distributed. Pretreatment with agrocin 84 had no apparent effect on the morphology or distribution of

Table 2 Comparison of the affinities of strains C58 and C58(NT-1) for habituated tobacco cells after preincubation in the presence or absence of agrocin 84

Strain	Applied bacteria associated with tobacco cells (%)	Average no. bacteria per tobacco cell $(\times 10^{-4})$
C58	93	14.5
C58 (agrocin-treated)	43.8	6.08
C58(NT-1)	15.5	2.96
C58 (NT-1) (agrocin-treated)	12.6	2.90

A. tumefaciens strain C58 and C58 (NT-1) were incubated in peptone broth for 12 h in the presence of $^{32}PO_4$ (5 μCi ml^{-1}). The cells were collected and washed 4 times in iso-osmotic phosphate buffer, *p*H 7.4 (0.155M NaH_2PO_4 adjusted to *p*H 7.4 with 0.103 M Na_2HPO_4). Each strain was then incubated in the presence and absence of agrocin 84 and inoculated into suspension cultures of habituated tobacco cells (see legend to Table 1). After 4 h aliquots of the infected tobacco cultures were layered over Ficoll (15% w/v in phosphate buffer) and centrifuged at 1,800*g* for 1 h. In such conditions 'free' bacteria are pelleted and the tobacco cells remain packed at the Ficoll interface. Approximately the same values were obtained in separate experiments in which separation of plant and bacterial cells was achieved by filtration through siliconised glass fibre filter paper disks. Controls utilised labelled bacteria incubated in plant growth medium in the absence of tobacco cells. Tobacco cells were stained with Trypan blue and counted using a haemocytometer. The specific activities of the bacteria were monitored from turbidity measurements and radioactivity determinations after Millipore filtration.

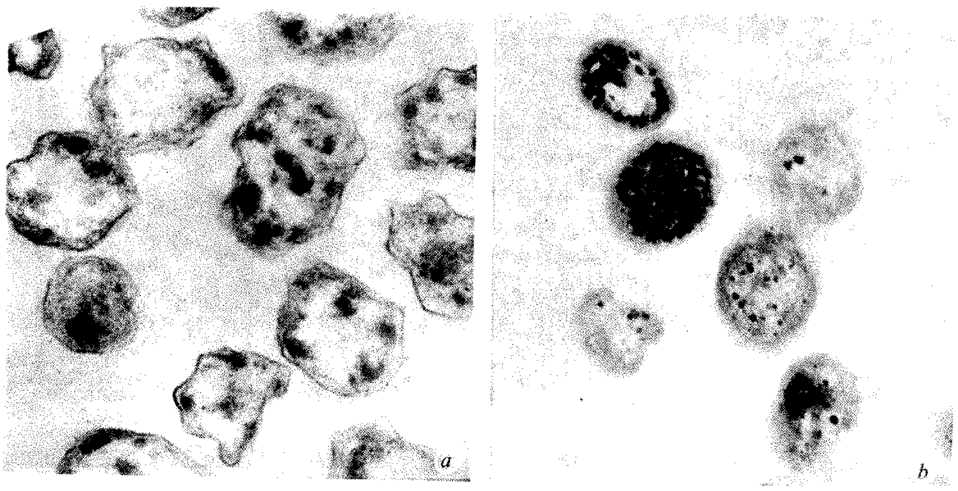

Fig. 2 Electron micrographs of transverse sections through *A. tumefaciens* C58 bacteria. *a*, Normal pathogenic C58 cells, ×2,780; *b*, C58 cells after a 2 h exposure to agrocin 84, ×2,780. The bacteria were prefixed in glutaraldehyde (2% v/v), post-fixed in osmium tetroxide (1% w/v) and embedded in Epon-araldite. Sections were stained with uranyl acetate followed by lead citrate.

these bacteria. In striking contrast however, the pathogenic C58 bacteria exhibited a marked tendency to cluster together and isolated cells were rarely observed. The bacteria did not apparently cluster around lesions in the host cell wall, but in some cases the entire surface appeared to be enveloped by the bacteria and in others localised areas were densely populated (Fig. 1*a*). Furthermore, the rod-shaped cells were orientated such that one end was embedded in the plant cell surface (Fig. 1*b*) and often a halo of diffuse material was observed around these bacteria. We do not consider that the observed polar attachment of bacteria to host is an artefact of the dehydration and fixation procedures as a previous cytological study of plants infected *in vivo* with *A. tumefaciens* showed the same phenomenon[14] and this is the mode of attachment of members of the closely related genus *Rhizobium*[20] to the tips of root hairs of their plant hosts[21].

The effect of agrocin 84 on strain C58 was immediately obvious; clustering was still apparent, but the majority of the bacteria were spherical and paired (Fig. 1*c*). As agrocin 84 is bacteriostatic (data not shown), the observation that the agrocin 84 treated cells were often located in pairs on the surfaces of the host suggested that bacteria which had entered the division cycle were unable to complete the process after the additon of agrocin 84. The further observation that these cells had rounded up suggested that agrocin 84 may act by disrupting the integrity of the cell wall.

To compare the anatomical features of normal and agrocin-treated bacteria, thin sections of these cells were examined under the transmission electron microscope. The electron micrographs shown in Fig. 2 are representative of our observations: untreated pathogenic cells of strain C58 were indistinguishable from cells of strain C58(NT-1) preincubated in the presence or absence of agrocin 84 and in all cases the cell envelopes were clearly defined. In contrast, the envelopes of agrocin-treated C58 cells were, when present, ill-defined and fragmented. Additionally, only these agrocin-treated cells contained large, electron dense granules and thus we suggest that agrocin 84 interferes with the synthesis of the cell wall rather than acting on preformed structures and that the granules may represent a build up of cell wall precursors.

The data presented here support the hypothesis that the cell surfaces of strain C58 and C58(NT-1) are different and that this difference is due to the presence of the tumorigenic plasmid (data not shown and ref. 2). The Ti plasmid of strain C58 seems to code for enzymes involved in the synthesis of surface components, believed to be capsular lipopolysaccharides[22], which mediate the specific attachment of bacterium to host. It is possible that these same components are also directly responsible for sensitivity to agrocin 84, but we consider this unlikely. Sensitivity to agrocin 84 is strictly dependent on the nutritional status of strain C58 and growth media which promote the synthesis of capsular lipopolysaccharide completely inhibit agrocin 84 sensitivity (data not shown). Furthermore, amongst the nopaline-utilising strains of *A. tumefaciens*, some pathogens are insensitive to agrocin 84 and conversely, some non-pathogens retain sensitivity (data not shown and ref. 13). Thus the correlation between pathogenicity and agrocin 84 sensitivity may be fortuitous rather than direct. We propose that agrocin 84 prevents tumorigenesis by causing the fragmentation or loss of the cell envelope and thereby impairing the ability of the bacterium to transfer tumorigenic DNA to its host. As agrocin 84 sensitivity is plasmid-coded in strain C58, the subsequent loss of this plasmid from cells incubated in the presence of this antibiotic is a requirement for survival.

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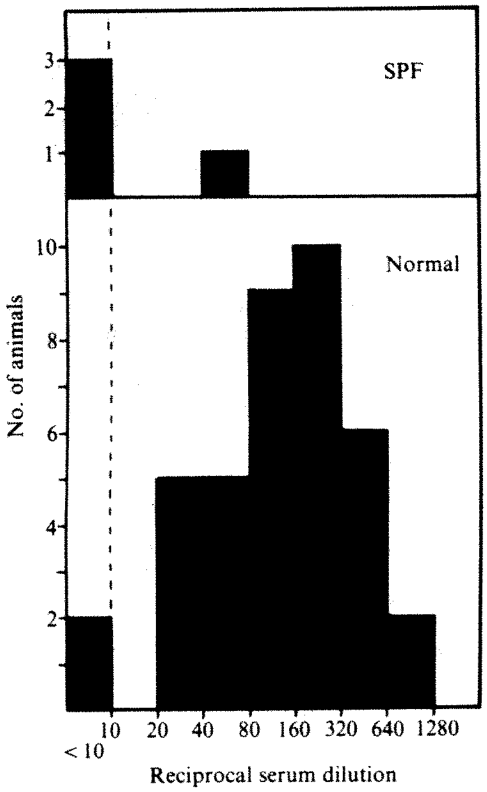
Neutralisation of respiratory syncytial virus by cat serum

RESPIRATORY SYNCYTIAL (RS) virus has been identified as a major cause of lower respiratory infection in infants, and the epidemiology of RS virus infection is characterised uniquely by annual winter outbreaks in the infant population and by antigenic stability of the virus[1]. The initial isolation of RS virus was from a captive chimpanzee[2] and more recently an antigenically similar virus has been associated with respiratory disease in cattle[3–5]. The bovine and human RS viruses, although antigenically similar, can be differentiated by their different host range *in vitro*[6]. The bovine RS virus had a wider host range, multiplying in bovine, hamster, swine and primate cells, whereas the human RS virus multiplied only in bovine and primate cells. We have observed recently, however, that human RS virus (but not bovine RS virus) can multiply efficiently in secondary cultures of feline embryo cells. The cytopathology induced by RS virus infection of feline embryo cells was similar to that described previously for African green monkey (BS-C-1) cells[7]: the surfaces of infected cells were seen to be covered by long slender filaments when viewed by scanning electron microscopy or by immunofluorescent staining (C.R.P. and J. E. Parry, unpublished data). The susceptibility of feline embryo cells to RS virus, and an observation that the sera of some laboratory cats in the USA inhibited RS virus (R.M. Chanock, personal communication), suggested that cats might be susceptible to RS virus infection. We therefore examined a random sample of cat sera, and we show that antibody to RS virus is widespread in domestic cats in Britain.

Fig. 1 Histogram of RS virus-neutralising activity in normal and SPF cats. Approximately 10^4 plaque-forming units of the RSN-2 strain of RS virus were mixed with twofold serial dilutions of each serum, and incubated at 4 °C for 18 h. The infectivity remaining in each tube was assayed by titration on BS-C-1 monolayers. The percentage reduction of infectivity was determined by plaque counting after a 7-d incubation at 31 °C under 0.9% (w/v) agar overlay and plotted against serum dilution. The dilution producing a 50% reduction of infectivity was determined graphically for each serum.



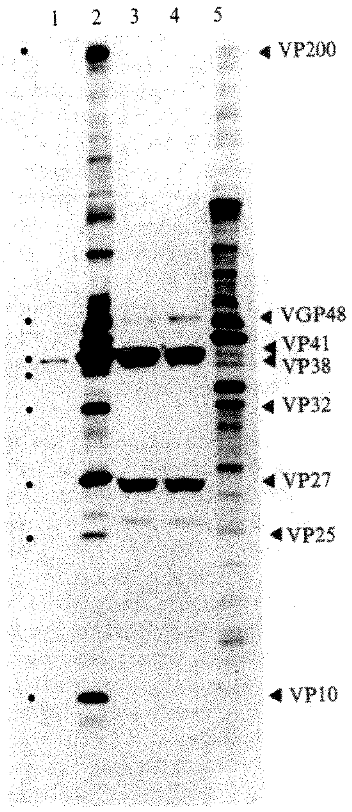


Fig. 2 Autoradiogram showing virus polypeptides precipitated by feline and bovine sera. Serum was incubated with ^{35}S-labelled RS virus-infected cells or a polyethyleneglycol (PEG) extract prepared from them for 1 h at 37 °C. Antibody–antigen complexes were precipitated with formalin-fixed heat-killed *S. aureus*[10]. Washed *S. aureus* precipitates were incubated at low *p*H to break antigen–antibody bonds. Viral antigens released into the supernatant were run on SDS-polyacrylamide gels (5.5–12% acrylamide gradients). Track 1, immunoprecipitate, cat serum + PEG extract of RS virus-infected cells, showing VP41. This band was absent when uninfected cells were used. Track 2, control. Radiolabelled RS virus-infected cells. Track 3, immunoprecipitate, bovine serum + PEG extract of RS virus-infected cells. Track 4, immunoprecipitate, bovine serum + RS virus-infected cell pellet. Track 5, control, radiolabelled uninfected cells.

Thirty-nine cat sera were obtained, representing a random selection of animals ranging from 2 months to 10 years old, 19 of which had been collected in 1975–6 and 20 in 1977–8. Four sera from specific pathogen-free (SPF) animals were also examined. The neutralising activity of these 43 sera was assayed as described in the legend to Fig. 1, and the 50% neutralisation end-points were determined graphically by plotting percentage recovery against serum dilution. The RSN-2 strain of RS virus[7] was used as the test virus. The results are presented in Fig. 1. Thirty-seven of the thirty-nine sera from normal cats had neutralising activity, with a geometric mean titre of 1/147, and only two were negative (<1/10). Three of the four sera from SPF cats, on the other hand, were negative (<1/10) and the one positive serum had a titre of 1/50 (approximately threefold less than the mean titre of the normal group). These results show clearly that the majority of the sera of domestic cats in this sample were able to neutralise the infectivity of RS virus. There was no obvious correlation between the level of neutralising activity and age of the animals, and the titres of the sera collected in 1975–6 and 1977–8 were distributed similarly. The greater frequency of negative sera from SPF animals (0.75 compared with 0.05 for normal animals) suggests that the neutralising activity represented an immune response.

This conclusion was supported by the result of an experiment in which extracts of ^{35}S-methionine-labelled infected and uninfected BS-C-1 cells were mixed with either a high titre convalescent bovine anti-RS virus serum, or one of the cat sera with

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high neutralising activity (1/768). The method of radiolabelling was as described previously[8,9]. Antigen–antibody complexes were precipitated with fixed *Staphylococcus aureus* as described in the legend to Fig. 2. The bovine anti-RS virus serum precipitated VGP48, VP41, VP38, VP27 and VP25, but not VP200, VP32 or VP10 (tracks 3 and 4 in Fig. 2). Significantly, no polypeptides corresponding to the high molecular weight glycopolypeptides described as RS virus-specific by Levine[11] were observed, confirming our previous interpretation[8,9] that these polypeptides were host-derived. Figure 2, track 1, shows that the cat serum possessed antibody able to combine with a viral protein which electrophoresed with the mobility of VP41, the nucleoprotein of RS virus[8,9]. No corresponding band was observed when the serum was reacted with uninfected cells (data not shown).

These observations suggest that most, if not all, domestic cats in this locality have experienced RS virus infection. The presence of neutralising activity in the sera of cats of all ages (and in one of the four SPF animals) suggests that cats may be highly susceptible to RS virus infection, and that they are exposed to infection early in life. It remains to be determined whether illness is associated with infection, and whether the virus inducing the immune response is transmitted horizontally among cats. It is possible that a feline RS virus exists, but as yet none has been isolated. The relevance of these observations to the human disease pattern is a question which merits further investigation.

We have not been able to detect RS virus antibody, however, by indirect immunofluorescent staining of acetone-fixed RS virus-infected cells using pooled and absorbed cat sera and an FITC-conjugated rabbit anti-cat globulin (Nordic). It would appear that the immunoprecipitation of radiolabelled viral protein is the more sensitive method for detection of RS virus antibody in cat serum.

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HLA-DR specific human suppressor lymphocytes generated by repeated *in vitro* sensitisation against allogeneic cells

THE HLA complex is composed of a set of genes closely linked on chromosome 6, which may have important functions in the regulation of the immune response. The HLA-A, -B and -C loci control serologically defined antigens present on most cells. The HLA-D region controls two groups of determinants: (1) HLR-DR (DRw1 to DRw8) antigens, restricted to B lymphocytes, detected by serology and equivalent to the I region-associated antigens (Ia) of the mouse; (2) HLA-D stimulating products (Dw1 to Dw11) defined by mixed lymphocyte reactions (MLR). Sensitisation of human lymphocytes with irradiated allogeneic stimulatory cells *in vitro* produces primed lymphocytes[1–5]. These *in vitro* primed lymphocytes develop an accelerated secondary proliferative response when restimulated by the specific priming cell or by any cell possessing HLA-D region products identical to the priming cells. Familial and population studies[6,7] show this secondary proliferation (MLR II or PLT) to be more correlated to HLA-DR antigens serologically detected than to D specificites defined by HLA-D typing (MLR I). It has also been suggested that, within informative families bearing haplotypes with unusual associated D-DR products, DR antigens and HLA-D stimulating products could be distinct determinants[7]. However, absolute proof—a recombination between these two products—is still lacking. The accelerated proliferative capacity of primed lymphocytes represents a function which depends on the allogeneic contact; we have investigated the possible induction of suppresor cells by repeated *in vitro* sensitisation. We show that lymphocytes sensitised twice '*in vitro*' behave as active suppressors of the allogeneic response and are specific for the HLA-DR antigens presented by the stimulator lymphocytes.

Table 1 Decrease in the specific proliferative response of the twice-primed PLT cells (PLT II)

Responding PLTs: anti DRw1 PLT I (c.p.m.)*	 PLT II (c.p.m.)*	Restimulating cells No.	 HLA-DR	 % Suppression
620	1,268	1	2/– NC	—
24,678	17,768	2	2/1 SC	.29
15,861	8,542	3	2/1	47
21,626	16,267	4	1/1	25
15,383	10,939	5	1/5	29
19,528	11,257	6	2/1	43
8,224	9,781	7	7/7	—
6,316	5,790	8	5/7	9
6,049	7,628	9	2/7	—
12,111	14,763	10	7/3	—
8,728	10,194	11	5/–	—

PLT I (anti DRw1 was produced within a normal family by sensitising *in vitro* 10×10^6 lymphocytes (isolated by Ficoll–Isopaque gradient) from the father DER. F (DRw2/–) with 10×10^6 2,500 rad γ-irradiated cells from his child DER. C (DRw2/DRw1) in 20 ml RPMI 1640 with 20% normal pooled human plasma and antibiotics (100 mg ml^{-1} streptomycin, 100 IU ml^{-1} penicillin), in plastic culture vials (Falcon 3013). They were incubated at 37°C in a 5% CO_2 air atmosphere for 10 d. PLT II was produced by sensitising a second time *in vitro* 10×10^6 PLT I cells with 10×10^6 irradiated cells from the same stimulator over 7 d. PLT testing was carried out in V-bottom plastic culture microtrays: 5×10^4 responders in 0.05 ml and 5.10^4 stimulators in 0.1 ml were added in each well. 48 h later cultures were pulse-labelled for 18 h using ^{3}H-thymidine (2 μCi per well) (specific activity, 1 Ci $mmol^{-1}$) and counted in a liquid scintillator. HLA-DR antigens were determined serologically and by PLT testing. Per cent suppression was calculated using the formula:

$$\%\ \text{Suppression} = \frac{1 - \text{test c.p.m. median value}}{\text{control c.p.m. median value}} \times 100$$

* Median values of three cultures.
† NC, negative control; SC, specific control.

The first indication that a suppressive activity can be detected in secondary cultures comes from the observation that twice-sensitised PLT cells (PLT II) give a lower accelerated proliferative response when restimulated by specific cells than classical PLT sensitised only once (PLT I). The kinetic study of this secondary proliferation has clearly shown that the proliferation was decreased and not delayed (data not shown). The proliferative response of PLT DER AC (PLT I) (an intrafamilial PLT generated against a DRw1-bearing haplotype) was compared with the response of the same PLT sensitised twice (PLT II) by the same priming cell (Table 1). The proliferative response of PLT I (anti DRw1) was significantly higher than the response of PLT II against DRw1-positive cells. As expected, both PLTs give the same low response against DRw1-negative cells. These results suggest that the lower specific response obtained with the twice-primed PLT (PLT II) is due to (1) depletion of the specific

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responding cells, or (2) to the generation of active suppressor cells after multiple *in vitro* restimulations. The latter hypothesis has been tested in a three-cell assay.

The three-cell assay is a proliferative assay; the three co-cultured cells were unprimed responding cells, irradiated stimulating cells and possible suppressor cells (responder cells primed twice and γ irradiated). The proliferative responses in the presence or absence of the suppressor cells were evaluated and compared on day 7 of culture. The results of experiments of this kind are shown in Tables 2–5. Addition of twice-primed irradiated cells (PLT II) in a three-cell assay induced an active suppression in some combinations. The specificity of this suppression was analysed using well typed unrelated and informative familial cells. It was first observed that a PLT II anti-DRw7 in family DEG (Table 2) was only suppressive when added in the combinations where the stimulating cells possess 'a' haplotype which codes for the DRw7 antigen. In the same experiment the response against an unrelated stimulating cell, GIL, DRw7/DRw7, was also suppressed. The specificity of the suppression in this and other families (data not shown) seemed to segregate with an HLA haplotype.

A more precise analysis of the specificity of the suppression was carried out by using an HLA-B/D recombinant family (BAR) in a three-cell assay (Table 3). PLT II anti-DRw7 cells were added in combinations using all the members of the family BAR as stimulators. A specific suppression for the DRw7-bearing 'b' haplotype was observed within this family. The combination with the recombinant child C1 who has only inherited the HLA-D part of the b haplotype was also suppressed. The specificity of the suppression seems to be HLA-D region specific.

The apparent specificity of the suppression for the HLA-D-region products could involve either DR antigens or D products—if these are distinct determinants. However, in familial cases where an unusual association between HLA-D and DR specificities occurs on one HLA haplotype, the respective role of the two 'different' products could be studied separately. An example is shown in Table 4: in family LIS an unusual haplotype was recognised previously, in which DRw1 is associated with Dw4 (instead of the usually observed DRw1–Dw1 association). A PLT II twice primed against an haplotype DRw1 Dw1 was added in combinations where different stimulators were used, either DRw1 Dw1 or DRw1 Dw4 bearing cells. A specific suppression for the DRw1 antigen of the stimulator was observed to be independent of the associated Dw specificity.

Experiments with lymphocytes primed against two DR specificities were also carried out. These lymphocytes were discriminative bispecific PLT reagents. Using repeated sensitisations with one or the other specificities, we sought to establish whether they could independently generate suppressors specific for one or the other DR antigen. For instance, DRw2/DRw2 lymphocytes were primed against cells possessing DRw5/DRw7 antigens for 10 days (bispecific PLT I) and co-cultured for 7 more days with either a DRw5/DRw5 or DRw7/DRw7 stimulator (PLTs II). The suppressive activity of these PLTs II was tested in a three-cell assay (Table 5). The bispecific PLTs restimulated by DRw5 antigens greatly suppressed the response against DRw5-positive cells, whereas the suppression of the response against DRw7-positive cells was lower. The opposite was observed when restimulation was carried out with DRw7 antigens. This suggests that there are two distinct classes of suppressors—one specific for DRw5-positive cells and the other for DRw7-positive cells.

Table 2 Suppression follows the HLA haplotype

Responder	Stimulators irradiated HLA haplotype	HLA-DR	Third cells added irradiated DEG C7 (c.p.m.)*	PLT II (c.p.m.)*	% Suppression
	DEG. F. a/b	7/3 SC†	16,164	6,966	57
	M. c/d	5/–	4,795	4,231	12
	C1 a/c	7/5	20,820	5,394	74
	C2 a/c	7/5	17,996	5,461	69
DEG. C7	C3 a/c	7/5	13,295	5,117	61
DRw 3/–	C4 a/d	7/–	13,181	3,934	70
	C5 b/c	3/5	3,844	6,684	—
	C6 b/c	3/5	3,060	3,286	—
	C7 b/d	3/– NC	740	1,412	—
	GIL.S	7/7	13,716	7,268	47
	SCH.D	1/1	14,360	16,488	—

Culture conditions were as reported in Table 1. PLT II was produced as follows: responder, DEG C7 (DRw3/–), and stimulator irradiated father, DEG. F (DRw3/DRw7) were co-cultured for 10 d (PLT I). These cells were restimulated for 7 d with an irradiated homozygous cell (DRw7/DRw7) and gave PLT II cells. Three-cell assays were carried out with: (1) responder cell unprimed, DEG. C7 (DRw3/–) (5×10^4 cells); (2) irradiated stimulating cells with different DR specificities (5×10^4 cells); (3) third irradiated cells either PLT II or DEG (DRw3/–) (5×10^4 cells). The proliferative response was evaluated on day 7 of culture, and the per cent suppression calculated as in Table 1.

* Medium values of three cultures.

† SC, specific control; NC, negative control.

Table 3 Suppression follows the D part of a recombinant HLA-B/D haplotype

Responder	Stimulators irradiated		haplotypes	HLA-A	B	DR	Third cells added irradiated LER (c.p.m.)*	PLT II anti-DRw7 (c.p.m.)*	% Suppression
	BAR.	F	a	28	14	–	34,442	15,192	56
			b	30	13	7			
		M	c	2	27	1	40,928	38,202	7
			d	3	35	5			
		C1	ab	28	14	/7	28,107	20,789	27
			c	2	27	1			
LER.		C2	b	30	13	7	45,489	30,379	34
DRw 2/2			c	2	27	1			
		C3	a	28	14	–	26,634	29,195	0
			b	2	27	1			
	VAL.	M	x	25	15	5	17,316	5,036	71
			y	1	44	7			
	LER.	C	z	3	7	2	1,412	1,817	—
			w	3	7	2			

PLT I produced with LER (DRw2/DRw2) as responder, and VDL M (DRw5/DRw7) as irradiated stimulator gave an anti DRw5 + DRw7 (PLT I) after 10 d. These cells sensitised a second time (7 d) against an homozygous irradiated cell (DRw7/DRw7) gave the PLT II cells. Three cell assays were carried out: (1) responder cell unprimed (LER DRw2/DRw2); (2) irradiated stimulating cells from members of family BAR. (in this family child C1 is a known HLA-B/D recombinant; (3) third irradiated cells, either PLT II (responder primed twice) or LER (DRw2/DRw2). The proliferative response was evaluated on day 7 of culture as for Table 2.

* Medium values of three cultures.

Table 4 PLT II cells specifically suppress the response to DR antigens

Responder	Stimulators irradiated	HLA haplotypes	DR	D	Third cell added irradiated DER. F (c.p.m.)*	PLT II anti-DRw1 (c.p.m.)*	% Suppression
	NC† 1	*a/b*	2/–	2/–	2,423	2,508	
	SC 2	*a/c*	2/1	2/1	43,594	8,024	81
	3	*a/c*	2/1	29,055	10,525	63	
	4	*a/b*	2/1	2/4	44,502	12,507	72
	5	*b/c*	1/5	4/5	38,018	12,911	66
DER F DRw w/–							
	6		1/1	1/1	30,355	10,170	66
	7		7/7	7/7	42,496	30,237	29
	8		5/7	5/7	30,853	28,144	9
	9		2/7	2/7	31,572	24,581	22
	10		5/–	5/–	31,674	37,555	—

PLT I produced with DER. F (DRw2 Dw2/–) as responder and DER. C, (DRw2 Dw2/DRw1 Dw1) as irradiated stimulator, gave after 10 d an anti-DRw1 PLT I; sensitised a second time, with the same irradiated stimulator for 7 d it gave PLT II cells. Three-cell assays were carried out with: (1) responder cell unprimed: DER F (DRw2 Dw2/–); (2) irradiated stimultaing cells from family members and unrelated donor's cells, (3) third irradiated cells, either PTL II (responder primed twice) or DER F, (DRw2 Dw2/–). HLA-D typing was carried out using HLA-D homozygous typing cells (7th Histocompatibility Workshop cells) in a standard micro MLR assay. The results were double normalised (according to the method of Ryder *et al.*[18]. DR determinants were as for Table 1.

* Medium values of three cultures.

† NC, negative control; SC, specific control.

Table 5 Suppressive activity of PLTs directed against two DR specificities and primed twice (PLT II), tested in a three-cell assay

Responder	Irradiated stimulating cells No.	DR	Control (LER 2/2) (c.p.m.)	PLT.II (c.p.m.)	Third irradiated cell added A % Suppression	PLT II (c.p.m.)	B % Suppression
	1	1/5	40,928	25,650	38	38,202	7
	2	2/5	15,700	8,878	44	17,840	—
	3	5/5	25,726	4,038	85	16,795	35
	4	3/5	30,006	9,287	70	15,947	52
	5	–/5	24,875	8,936	65	20,866	17
	6	5/5	38,093	9,212	76	27,355	29
	7	5/5	25,622	9,093	65	19,644	24
LER DRw2/DRw2	8	–/7	34,442	23,963	31	15,192	56
	9	1/7	28,107	34,439	—	20,789	27
	10	1/7	45,489	38,879	15	30,379	34
	11	3/7	16,125	22,330	25	18,014	32
	12	7/7	26,290	20,579	22	14,458	46
	13	–/7	22,983	25,749	—	15,363	34
	14	1/–	26,634	28,250	—	29,195	0
	15	–/3	30,350	26,801	12	27,682	9
	16	–/3	20,523	30,311	—	24,712	—
	17	3/3	20,807	27,683	—	26,702	—
	18	2/3	26,898	17,549	35	25,824	4
	Negative control		2,770	1,654		2,940	

Bispecific PLT I was produced with LER DRw2/DRw2 as responder and VDL M (DRw5/DRw7) as irradiated stimulator. PLTs II were produced by sensitising PLT I cells a second time with either cells GIL (DRw7/DRw7) = PLT II A or cells MAI (DRw5/DRw5) = PLT II B for 7 d. The three-cell assay was carried out as follows with: (1) responder cell unprimed· LER (DRw2/DRw2), (2) γ-irradiated stimulating cells with different specificities (DRw5, DRw7, DRw3 ...); (3) third γ-irradiated cell: either PLT II A or B (responder primed twice, see Table 2) or LER (DRw2/DRw2).

These experiments indicate that lymphocytes which have been sensitised twice *in vitro* contain active suppressor cells, capable of suppressing 30–80% of the allogeneic proliferative response of unprimed cells. These *in vitro*-generated suppressor cells are radioresistant and specific for the HLA-DR antigens or closely associated structures presented by the stimulator lymphocytes. The suppressive activity of lymphocytes primed against two HLA-DR antigens seems to be induced by two distinct populations of suppressor cells. These suppressors behave (at least for the specificity of the suppression) in the same way as described for lymphocytes from a multiparous woman[8,9] which were spontaneous suppressors of the response against HLA-D-region products of her husband, and were also radioresistant. However spontaneous suppressors specifically restricted to HLA-A specificities have also been reported[10].

In vitro-generated suppressor cells are probably T lymphocytes, as only T cells are known to proliferate in MLR; however, there is no direct evidence. Cell-mediated lymphocytotoxicity cannot explain the suppression phenomenon for at least two reasons. (1) In our system, cell division was inhibited by irradiation, which prevents reactivation of the cytotoxicity of the twice-primed lymphocyte population[11]. (2) In contrast to the suppression observed here, the specificity of the lymphocytotoxicity has not so far been shown to be directed against HLA-DR antigens[12]. An antigenic competition, even though unlikely, cannot be definitely excluded. We shall investigate a possible HLA restriction phenomenon between the responder and the suppressor lymphocytes as well as a possible suppression mediated by soluble factors.

These HLA-DR-specific *in vitro*-generated human suppressors are probably similar to the spontaneous suppressors described *in vivo* after pregnancy[8,9]. On the other hand, this allogeneic phenomenon seems to be different from other suppressors reported in human, such as those induced by mitogenic stimulation[13–16] and observed in various diseases[17]. The suppression described here can be considered as a balanced phenomenon involving interaction between different lymphocyte populations such as 'helper cells', responsible for the allogeneic proliferative response and 'suppressor cells', antagonists of helper cell activity. This phenomenon might be of great importance during pregnancy and in allograft tolerance.

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Postnatal cerebellar cells from staggerer mutant mice express embryonic cell surface characteristic

MORE than 100 mutations affecting neurological function have been described in mice[1]. In one of these, staggerer[2,3], the development of normal synaptic relationships between cerebellar granule cells and Purkinje cells is disturbed[4–6]. In a search for abnormalities in the cell surface of staggerer cerebellar cells, we have measured cell agglutination in the presence of the plant lectins concanavalin A (Con A) and wheat germ agglutinin (WGA). We report here that cell surface properties necessary for WGA-induced agglutination are abnormal in the mutant cerebellar cell population whereas those necessary for Con A-induced agglutination are not. These results are consistent with the persistence of a characteristic of the embryonic cerebellar cell surface.

Wild-type and staggerer animals maintained on A C57−+*d se*/*sg* ++ inbred background were obtained from the breeding colony of our department[7]. Homozygous staggerer offspring were identified by their black coat and neurological symptoms[2]. All mice were decapitated on the seventh and tenth days after birth (P7, P10), P7 being the earliest age when staggerer animals could be identified. Wild-type littermates were identified by their dilute coat colour. Cerebella were removed and single cell suspensions were prepared by dissociation with trypsin-DNase followed by gentle trituration[7,8]. Cell yield was $2\text{–}3\times10^6$ per P7 staggerer cerebellum and $1\text{–}2\times10^7$ per P7 wild-type cerebellum.

To allow recovery from brief exposure to trypsin during dissociation, cells were preincubated in Ham's F12 medium supplemented with horse serum (2%, Microbiological Associates) for 6 h at 35.5 °C. The serum was removed by washing twice with phosphate-buffered saline (PBS, 600 r.p.m., 5 min, 4 °C), and the cells were resuspended (1×10^6 cells per ml of PBS) for agglutination assays.

As described previously[10] (summarised in Table 1), early embryonic wild-type cerebellar cells are highly agglutinable with both Con A and WGA. This agglutinability is lost, however, after about embryonic day 16, such that postnatal cerebellar cells require very high concentrations of the lectins for agglutination. In contrast, P7 and P10 staggerer cerebellar cells were highly agglutinable with WGA. Hapten-specific inhibition of staggerer cerebellar cell agglutinating activity (%) was measured in quadruplicate with WGA (100 μg ml^{-1} in PBS) preincubated (15 min, 20 °C) with the inhibitor. In those conditions, WGA-induced agglutination of P7 staggerer cerebellar cells was inhibited completely by *N*-acetyl-D-glucosamine (0.40 mM), *N*-*N*-di-*N*-acetylchitobiose (0.04 mM), chitin hydrolysate (prepared by Rupley's method[11] and approximately 0.02 mM, 1% w/v; hydrolysate demonstrated by paper chromotography to consist primarily of di- and trisaccharides) and ovomucoid (0.02 mg ml^{-1}). Agglutinating activity was not reduced by more than 50% by D-glucosamine (50.0 mM), D-galactose (50.0 mM), *N*-acetyl-D-galactosamine (50.0 mM) or methyl-α-D-mannoside (50.0 mM). These results agree with the reported carbohydrate binding specificity of WGA[12,13].

Table 1 Agglutination of staggerer and wild-type mouse cerebellar cells with Con A and WGA

Genotype	Age	WGA	Con A
Wild-type C57BL/6J	E13	50	200
	P0	450	800
	P3	>500	>1,000
	P7	>500	>1,000
	P10	>500	>1,000
Wildtype +*d se*/+ *d se*	P7	>500	>1,000
	P10	>500	>1,000
Staggerer *sg* + +/*sg* ++	P7	55	>1,000
	P10	48	>1,000

Values for C57BL/6J embryonic animals are taken from ref. 10. Values for postnatal wild-type and staggerer animals are given as lectin concentration (μg ml^{-1}) required for half maximal agglutination (75%). For details of the assay method see refs 9 and 10 Agglutination assays were carried out in quadruplicate. Results given are averaged values from four sets of assays, each set having been carried out with animals from a separate litter. 20 staggerer animals and 10 wild-type animals were studied. The variability among quadruplicate assays of lectin concentration required for half maximal agglutination was less than 15 μg ml^{-1}.

Table 2 Recovery of P7 wild-type and P7 staggerer cerebellar cells from exposure to trypsin

Genotype		Con A (μg ml^{-1})	WGA (μg ml^{-1})
Wild-type	*a*)	150	50
+*d se*/ + *d se*	*b*)	>1,000	>500
	c)	160	65
Staggerer	*a*)	150	50
sg + +/*sg* ++	*b*)	>1,000	50
	c)	150	50

Lectin concentration (μg ml^{-1}) required for half maximal agglutination (75%). Agglutination assays were carried out in quadruplicate by the method of Burger[9] *a*, Agglutination immediately following dissociation of cerebellar tissue into a single cell suspension[7,8]. *b*, Agglutination of dissociated cells replaced in Ham's F12 medium supplemented with horse serum (2%, Microbiological Associates) for 6 h, at 35.5 °C before the assay. *c*, Agglutination of dissociated cells replaced in growth medium as above for 6 h followed by exposure to trypsin (crystalline, Worthington, 10 μg ml^{-1} in PBS) for 10 min at 37 °C. Subsequently ovomucoid (10 μg ml^{-1} in PBS) was added, the trypsin was removed by washing the cells three times in PBS (600 r.p.m., 5 min, 4 °C), and the cells were resuspended for agglutination assays. The variability among quadruplicate assays of lectin concentration required for half maximal agglutination was less than 15 μg ml^{-1}.

As with early postnatal wild-type cerebellar cells, cells derived from staggerer mice were not agglutinable with Con A at P7 or P10 (Table 1). They could be agglutinated only by high lectin concentrations or by brief proteolysis as described below. The latter agglutination was specifically inhibited by methyl-α-D-mannoside (2.0 mM) but not by other haptens, in agreement with reports of Con A binding specificity[12–14].

Interestingly, the cell surface abnormality in staggerer cerebellar cells was restricted to components involved in WGA-induced agglutination. In studies of wild-type cells from several brain regions, WGA-induced agglutination depended on the region of origin and age of the cells as well as the enzymatic treatment to which the cells had been subjected. Whereas cells from the embryonic cerebellum agglutinated with low concentrations of WGA[11], those from neural retina[15], midbrain, medulla and cerebral cortex agglutinated only immediately after trypsinisation[11]. The abnormality of the staggerer cerebellar cells could thus reflect the persistence postnatally of the early embryonic cell surface characteristics which result in high agglutinability by WGA.

This conclusion depends on the recovery by the cells from the effects of the trypsin used to prepare the cell suspension, and on the absence of large changes in the different cell populations caused by selective granule cell degeneration in mutant staggerer cerebellum. Evidence for the recovery of both wild-type and staggerer cerebellar cells from the effects of trypsin is presented in Table 2. Before replacing cells in growth medium for 6 h, P7 and P10 cerebellar cells collected from wild-type and staggerer animals were highly agglutinable with both Con A and WGA (Table 2*a*). This agglutination was hapten-specific, Con A-induced agglutination being inhibited exclusively by methyl-α-D-mannoside[12,16] (2.0 mM) and WGA-induced agglutination by *N*-acetyl-D-glucosamine After incubation in growth medium for 6 h, the Con A- and WGA-induced agglutinability of wild-type cells and the Con A-induced agglutinability of staggerer cells decreased markedly (Table 2*b*). To directly assess the response of recovered cells to trypsin, we subjected them to a second mild proteolysis. The result was a second increase in the Con A- and WGA-induced agglutinability of wild-type cells and in the Con A-induced agglutinability of staggerer cerebeller cells. A second exposure to trypsin did not further increase the WGA-induced agglutination of staggerer cells (Table 2*c*).

These results are consistent with the effects of brief exposure to trypsin on the response of several cell types to Con A and WGA. Trypsin reversed non-agglutinability but did not affect high agglutinability[16-19]. Trypsin also has a transient effect on 3T3 cells, BHK cells and chick embryo fibroblasts, which become refractory to increased lectin-induced agglutinability within 6 h of the removal of the trypsin[20-22].

At P7, the age with which we are chiefly concerned here, the granule cell population of staggerer is 50% smaller than that of wild-type cerebellum. The other major cell populations, Purkinje cells and glial cells, are not different for staggerer[24,25]. The three- to fivefold decrease in total cell yield we obtained for staggerer cerebellum was therefore due primarily to the selective loss of granule cells. To assess whether a smaller proportion of granule cells in the cell suspension influenced our agglutination results, we resuspended wild-type cerebellar cells at 3×10^6 instead of 1×10^6 cells per ml for agglutination assays. Agglutination of this cell suspension by Con A and WGA was identical to that reported in Table 1. It is therefore unlikely that dilution of other cell populations by the larger granule cell population in wild-type cerebellum exclusively explains the difference in WGA-induced agglutination found for staggerer cerebellar cells. This interpretation is strengthened by recent studies showing that lectin-induced agglutination can be used to separate out a subpopulation of undifferentiated cells from a mixed population of cloned embryonal carcinoma cells[26].

Because the agglutination assays were carried out in suspension, it is not possible to identify the cell type in the staggerer cell population which is the site of the abnormality in WGA-induced agglutination. We shall investigate this by electron microscopy of agglutinated cells and by histochemical studies of WGA-binding to frozen tissue sections.

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Erythroid bursts produced by Friend leukaemia virus *in vitro*

WHEN mouse haematopoietic cells are incubated in plasma clot or methyl cellulose cultures in the presence of erythropoietin (EP), at least two kinds of colonies are observed[1-4]. After 2 days, randomly distributed erythroid colonies (defined as a clone of eight or more benzidine-positive cells) appear[1,2], and after 3–8 d of culture with higher concentrations of EP, larger colonies arise in clusters or 'bursts'[3,4]. The cells of origin have been termed the colony-forming unit-erythroid (CFU-E)[1] and burst-forming unit-erythroid (BFU-E)[3,4], respectively. Erythropoiesis in mice is also increased considerably by the Friend virus complex[5,6], which has been studied mostly *in vivo* although there are systems for the study of erythroid transformation *in vitro* soon after infection[7-10]. Nooter and Bentvelzen[11], using methyl

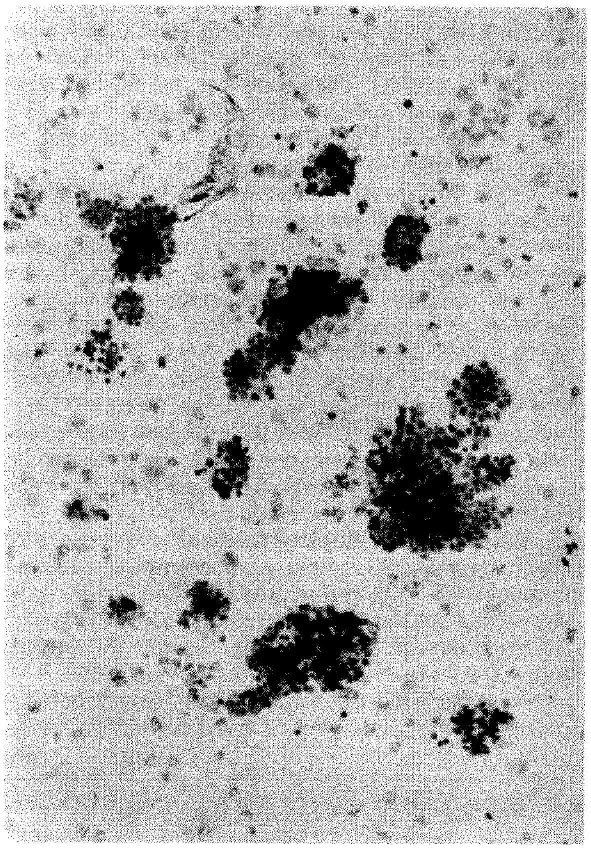

Fig. 1 Erythroid bursts produced by Friend virus infectious plasma. BALB/c mice were given three intraperitoneal injections (0.04 mg per g) of phenylhydrazine hydrochloride 62, 48 and 40 h before killing. When the mice had been killed, marrow cells were flushed from the tibias and femurs with sterile Hanks balanced salt solution (HBSS). The cells were washed twice with HBSS and collected each time by centrifugation. 4×10^6 nucleated cells were resuspended in 0.3 ml of a 1:20 dilution of Friend virus infectious plasma in alpha medium (7,500 spleen focus-forming units[18] and 1.5×10^5 XC plaque forming units[19]). This cell suspension was incubated at 4 °C for 1 h. The virus–cell mixture was then diluted to 2 ml to give the following final concentrations: bovine serum albumin, 1% v/v; penicillin, 100 IU ml^{-1}; streptomycin, 100 μg ml^{-1}; β-mercaptoethanol, 10^{-4}M; fetal calf serum, 30% v/v; beef embryo extract, 0.85% v/v; bovine citrated plasma, 10% v/v. This mixture was pipetted into triplicate 0.5 ml tissue culture wells (Disposo-Trays, Model 96-SC, Linbro) and incubated at 37 °C in humidified atmosphere of 5% CO_2–95% air. After 5 d of incubation, the clots in the wells were placed on glass slides, fixed with 5% glutaraldehyde and stained with 3,3′-dimethoxybenzidine and Harris haematoxylin[3].

cellulose cultures, showed that mouse marrow cells, previously incubated *in vitro* with purified Rauscher leukaemia virus, develop more erythroid colonies than untreated control cells. Clarke *et al.*[12], using the plasma clot method, observed erythroid colonies 2 d after incubation of mouse marrow cells with culture fluid from Friend virus infected monolayers of fibroblasts. These colonies were similar to EP-induced 2-d colonies in gross morphology and random distribution throughout the clot. Virus-induced bursts were not present in later stages of culture and erythroid colonies were not induced after incubation of marrow cells with plasma from Friend virus infected mice. We now report that when mouse marrow cells are incubated *in vitro* with infectious plasma or tissue culture fluids containing the Friend virus complex, a substantial number of erythroid bursts appear 5 d after the initiation of the cultures. The Friend virus complex consists of at least two biologically active viruses—the spleen focus-forming virus (SFFV) which is replication defective and essential for increased erythropoiesis *in vivo*, and a helper murine leukaemia virus (MuLV-F)[13,14].

The time elapsed between primary infection with Friend virus complex *in vivo* and the peak of haemoglobin synthesis *in vitro* is 4–5 d (ref. 15). The size of the peak depends on the dose of virus and the time after infection at which the cells are placed *in vitro*, but the time course of haemoglobin synthesis is constant. Although these results were obtained in liquid cultures and with ^{59}Fe which was incorporated into haem[15], Friend virus-infected marrow cells might be expected to produce benzidine-positive cells in semi-solid cultures within a period similar to that required for the wave of ^{59}Fe incorporation.

Marrow cells from mice treated with phenylhydrazine to make them anaemic, were incubated with a 1:20 dilution of Friend virus plasma and seeded in plasma clot cultures. A typical 5-d burst is shown in Fig. 1. We observed similar but fewer bursts with marrow cells from normal mice. In contrast to erythroid colonies that appear 2 d after the addition of EP, almost all colonies seen 5 d after the addition of Friend virus complex were in clusters resembling the bursts produced by EP[3,4]. Each cluster consisted of multiple colonies of erythroblasts containing from 8 to more than 100 cells. It has been shown that all the colonies of an EP-induced burst arise from a single cell[16]. Whether the Friend virus complex acts on the same cell that responds to EP with the formation of 5-d bursts remains to be determined.

As shown in Table 1, the number of 5-d bursts decreased progressively as the cells were incubated with increasing dilutions of infectious plasma. A constant association was observed between burst forming ability and focus-forming ability in two separate infectious plasmas and cell culture supernatants from 3 different cell lines producing SFFV. In contrast, burst forming activity was not detected in normal BALB/c mouse plasma or cell culture fluids from cell lines which produced only MuLV.

Two sets of results (Table 1 and Fig. 2) suggest that the development of erythroid bursts after the addition of Friend virus infectious plasma is a direct effect of virus on the marrow cells. First, heat treatment at 56 °C for 1 h rendered Friend virus infectious plasma incapable of inducing splenic foci *in vivo* and producing splenomegaly[5,6,15]. Heat treatment of the infectious plasma either at 80 °C for 3 min or at 56 °C for 1 h abolished the formation of 5-d bursts *in vitro* (Table 1). EP is heat stable[17] and similar treatment did not destroy its ability to induce erythroid colonies from mouse marrow cells after 2 d of culture in the plasma clot system (our unpublished results). Second, Friend virus infectious plasma was layered over a 5–20% linear sucrose gradient, and an EP preparation was layered on an identical gradient (Fig. 2). Both gradients were centrifuged and fractionated. Each fraction of the gradient containing infectious plasma was assayed for its ability to induce 5-d erythroid bursts, and for its content of SFFV and MuLV-F as defined by the spleen focus-forming assay[18] and XC assay[19], respectively. Most of the 5-d erythroid burst-forming activity, as well as XC plaque and spleen focus-forming activities, were recovered in the bottom half of the gradient. There was no activity at the top, where EP would have been located. Thus, erythroid burst formation was produced by an entity that sedimented with the known virus activities in Friend virus preparations. To detect EP activity, fractions of the EP gradient were tested for their ability to induce 2-d erythroid colonies. As expected, many colonies were produced by fractions in the top part of the EP gradient, but only small, background, numbers were produced by lower fractions.

Table 1 Relation of SFFV activity to production of 5-d erythroid bursts

Test preparation	Dilution	Contains SFFV	Contains MuLV-F	Average 5-d bursts per clot
	Expt 1			
M19 plasma	1:20	+	+	23
M19 plasma treated, 56 °C × 1 min.	1:20		ND*	0
M18 plasma	1:20	+	+	30
M18 plasma	1:40	+	+	20
M18 plasma	1:80	+	+	12
M18 plasma	1:160	+	+	9
M18 plasma	1:320	+	+	2
M18 plasma	1:640	+	+	1
M18 plasma treated, 80 °C × 3 min	1:20	–	ND	0
Normal BALB/c mouse plasma	1:20	–	ND	0
	Expt 2			
Tissue culture supernatants:				
SC-1† (M16)	Undiluted	+	+	11
FP52‡	Undiluted	+	+	23
EY26§	Undiluted	+	+	3.6
SC-1 (M16) treated, 56 °C × 60 min	Undiluted	–	ND	0
FP52 treated, 56 °C × 60 min	Undiluted	–	ND	0
EY26 treated, 56 °C × 60 min	Undiluted	–	ND	0
SC-1† MuLV-M	Undiluted	–	+	0
SC-1† MuLV-F	Undiluted	–	+	0
1902§ Nonproducer cells	Undiluted	–	–	0

In expt 1, marrow cell suspensions were prepared from mice pretreated with phenylhydrazine as in Fig. 1. The cell suspensions were mixed with the indicated test preparations and incubated as described in Fig. 1. M19, M18 and M16 refer to separate pooled plasmas prepared from Friend virus-infected mice as previously described[15]. In expt 2, marrow was prepared from normal BALB/c mice. EY26 cells are '1902' cells (nonproducer cells that contain SFFV genetic information[22], but do not release virus particles) which were superinfected with cloned murine leukaemia virus to rescue SFFV[22]. FP52 cells were developed by infection of mouse fibroblasts with Friend virus[21]. SC-1 cells[20] were infected with Moloney leukaemia virus obtained from the ATCC MD {SC-1 (MuLV-M)}; M16 Friend virus infectious plasma prepared as previously described[15] {SC-1 (M16)}; or M16 plasma at a dilution that is past the end point of SFFV {SC-1 (MuLV-F)}.

* ND, not determined

† SC-1 cells[20] infected with an infectious plasma preparation (M16), MuLV-Moloney or MuLV-Friend.

‡ A gift from Dr Alan Bernstein[2].

§ A gift from Dr David Troxler[22].

Thus, we have shown that when Friend virus complex is incubated *in vitro* with mouse marrow cells erythroid bursts develop 5 d later. Bursts can also be seen when the clots are removed on days 3–8, and the timing for the optimum number of bursts remains to be determined. The production of erythroid bursts after incubation of haematopoietic cells with infectious plasma provides an *in vitro* method for studying the interaction of the Friend leukemia virus(es) with the target cell(s). Various indirect *in vivo* approaches have been used to define the cells which are the targets for erythroid transformation by the Friend and Rauscher viruses. A direct *in vitro* approach is now possible.

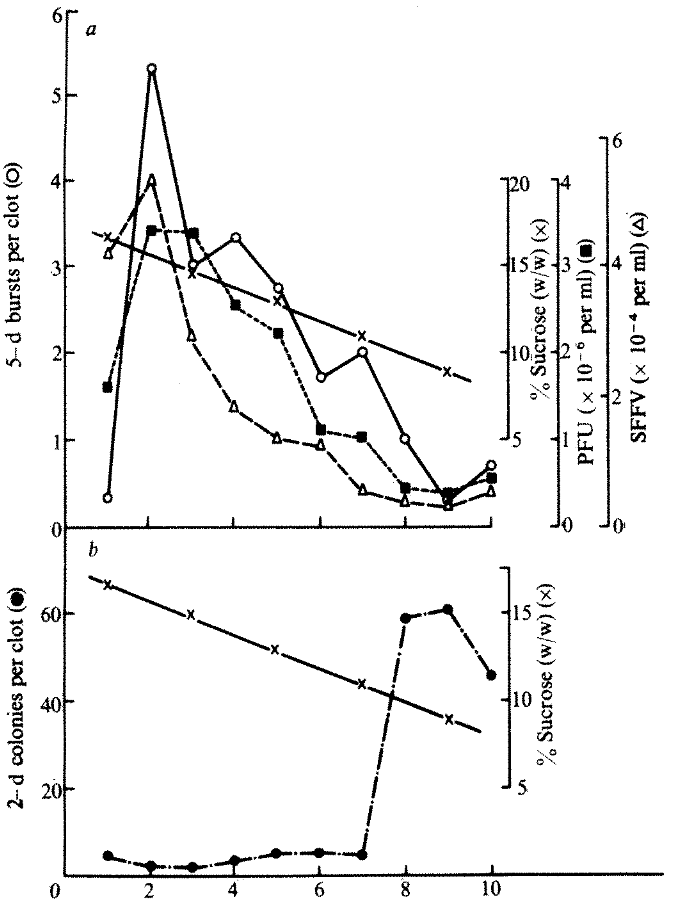

Fig. 2 Recovery of 5-d erythroid burst-forming activity after velocity gradient sedimentation of Friend virus infectious plasma. One ml of infectious plasma was layered over a 4.5 ml 5–20% w/v linear sucrose gradient and centrifuged at 45,000 r.p.m. for 7 min at 4 °C in an SW50.1 rotor. An erythropoietin preparation (EP, Step III, Connaught Medical Research Laboratories) was centrifuged on a similar gradient. Fractions of 0.5 ml were collected from the bottom of both gradients. Marrow cells from phenylhydrazine-treated mice were incubated as in Fig. 1 with each fraction which was diluted 1 : 10 with alpha medium and filtered through 0.45-μm filters. Plasma clots were examined for colonies or bursts at 5 d (*a*) and at 2 d (*b*). In (*a*) the fractions were tested in the spleen focus-forming[18] and XC plaque-forming assays[19].

and experiments are in progress to delineate these cells. This is the first demonstration of the production of erythroid bursts *in vitro* by the Friend virus complex. Because the effect of the virus in our system is quite large (each cluster consists of multiple large colonies) we have now a better means of studying the process of erythroid transformation by a murine virus. It should also be possible to develop a suitable *in vitro* assay for SFFV.

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Mutagenesis of Chinese hamster cells is facilitated by thymidine and deoxycytidine

ONE-WAY information transfer from nucleic acids to proteins is generally considered to ensure accurate cell self-duplication, in which errors occur by mutation[1]. However, it has been argued that self-duplication is a multimolecular process consisting of several potentially error-prone stages[2], and that mutation is a complex cellular process[3]. Moreover, it has been shown that error-prone DNA polymerases cause mutations in bacteriophage T_4 (ref. 4.5). We now report that mutations produced by *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), at two separate loci in Chinese hamster cells, are increased 3–10-fold when the cells are treated in the presence of low concentrations ($\sim 2\times 10^{-5}$ M) of thymidine (TdR) and deoxycytidine (CdR). We conclude that imbalanced nucleotide pools facilitate errors of replication of methylated DNA, and suggest that aberrant synthesis of macromolecules, facilitated by imbalances in precursor pools, plays an important part in the expression of genetic damage.

Figure 1*a* shows that in the presence of CdR or TdR (0.5–2.0 μg ml^{-1}) the frequency of ouabain-resistant (Oua^r) colonies induced by MNNG was increased 2–15-fold, the most consistent increase (2.9–3.0-fold) occurring in the presence of equal concentrations of TdR and CdR. Figure 1*b* shows that in the presence of CdR and TdR the frequency of 8-azaguanine-resistant (AzG^r) colonies induced by MNNG was consistently increased 2.7–4.3-fold. However, these nucleosides did not increase the yield of AzG^r or Oua^r colonies that arose spontaneously in the two experiments shown in Fig. 1. The spontaneous frequencies were $\sim 1\times 10^{-5}$ survivors for AzG^r and Oua^r colonies.

Figure 1*c* shows that cytotoxicity of the LD_{50} level of MNNG (1.0 μg ml^{-1}) was greatly increased when cells were grown and treated in dialysed fetal calf serum in the presence of as little as 0.5–2.0 μg ml^{-1} of TdR. This effect did not occur in medium containing the same concentrations of both TdR and CdR. In medium containing CdR in the absence of TdR, MNNG was ~20% less toxic than in medium to which no nucleoside had been added. In the absence of MNNG, CdR and TdR, separately, at concentrations of 0.5–2.0 μg ml^{-1} reduced the plating efficiency by only 7–17%.

The results shown in Fig. 1*a* and *b* are surprising, because cells possess controls to ensure that the DNA replication mechanism selects the correct nucleotides[6]. Therefore, it is necessary to inquire whether the colonies scored for Fig. 1*a* and

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b represent increased mutation frequencies, selective toxicity of MNNG for non-mutant cells, selective growth of mutants, decreased time for expression of the mutant phenotypes, or modification of MNNG or its cellular targets in the presence of CdR and TdR. Experiments designed to test these possibilities have been carried out and are summarised below. They will be reported in detail elsewhere.

AzG^r clones have been extensively characterised in previous studies in our laboratory[7,8]. Of 20 AzG^r colonies from V79 cells treated with MNNG in the presence of TdR and CdR (4.0 μg ml^{-1}), 70% showed the AzG^r, HAT (10^{-5}M hypoxanthine, 10^{-6} M aminopterin, 5×10 M TdR) sensitive (HAT^s) phenotype characteristic of hypoxanthine: guanine phosphoribosyl transferase (HGPRT) deficient mutants[7,8], 5% showed the AzG^r, HAT^r phenotype found in mutants containing HGPRT with an altered K_m for substrate[9,10], and 25% were unable to grow to confluence in either AzG or HAT. None of the isolates showed the AzG^s, HAT^r phenotype of the parental non-mutant cells. Therefore, we consider all the colonies that were scored for Fig. 1*b* to be mutants. Of 17 Oua^r colonies isolated, all remained completely resistant to 3 mM ouabain 8 and 35 d after isolation. Therefore, we consider the Oua^r phenotype to be characteristic of mutants containing a modified (Na^+, K^+)ATPase[11,12].

Both in the presence of CdR, or CdR plus TdR, and in their absence, cells treated with 0.5 μg ml^{-1} of MNNG underwent the same number of doublings (3–3.5) before exposure to ouabain or AzG. Moreover, we have measured the AzG^r colony frequency over 12 population doublings in cultures treated with the LD_{50} level of MNNG in the presence or absence of CdR, TdR and CdR plus TdR (2.0 μg ml^{-1}). In those experiments, the mutation frequencies in cultures grown in the presence of CdR and TdR remained three- to seven-fold higher than the frequencies obtained with MNNG alone. These findings show that the increased mutation frequencies produced by CdR and TdR are not the result of differences in expression time.

The doubling time (12.7±0.66 h) of AzG^r colonies isolated from cultures treated with MNNG in the presence of CdR plus TdR was not different from the doubling time (12.4±1.5 h) of AzG^s cells in medium containing CdR plus TdR (2.0 μg ml^{-1}). Therefore, the AzG^r mutants did not have a selective advantage over AzG^s cells in medium containing CdR and TdR.

In the absence of TdR, concentrations of CdR ranging over 0.5–2 μg ml^{-1} produced the same slight (<20%) reduction in MNNG cytotoxicity (Fig. 1*c*), showing that the CdR was not preventing the MNNG from reaching targets for cytotoxicity within the cells.

Moreover, DNA single-strand breaks produced by MNNG were measured using our alkaline sucrose-gradient procedure[13,14]; the extent and also the persistence of the breaks produced in the presence of the CdR plus TdR (2.0 μg ml^{-1} of each) or in the absence of the nucleosides were not significantly different. Therefore, neither CdR nor TdR prevented MNNG from reaching the DNA. Thus, we consider it most unlikely that the effects of the nucleosides on cytotoxicity and mutation result from modification of MNNG or its cellular targets before alkylation occurs. We must therefore conclude that MNNG-induced mutagenesis at two different loci (one, recessive, that involves purine nucleotide metabolism, and the other, dominant, that involves cation transport through the cell membrane) in Chinese hamster V79 cells, was increased three- to four-fold in the presence of 0.5–2.0 μg ml^{-1} of CdR plus TdR. Moreover, we have obtained similar results using *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine (ENNG) and ethyl methane sulphonate (EMS) as mutagens (A.R.P., H.P. and C.H., unpublished data).

The MNNG-induced mutation frequency increases with increase in the toxicity of MNNG in Chinese hamster cells[7,8,15,16], so the increased mutation frequency observed in the presence of TdR could have been associated with the greatly increased toxicity with TdR (Fig. 1*c*). However, when both CdR and TdR were present, the TdR sensitisation of the cells to the cytotoxicity of MNNG was abolished (Fig. 1*c*), although the

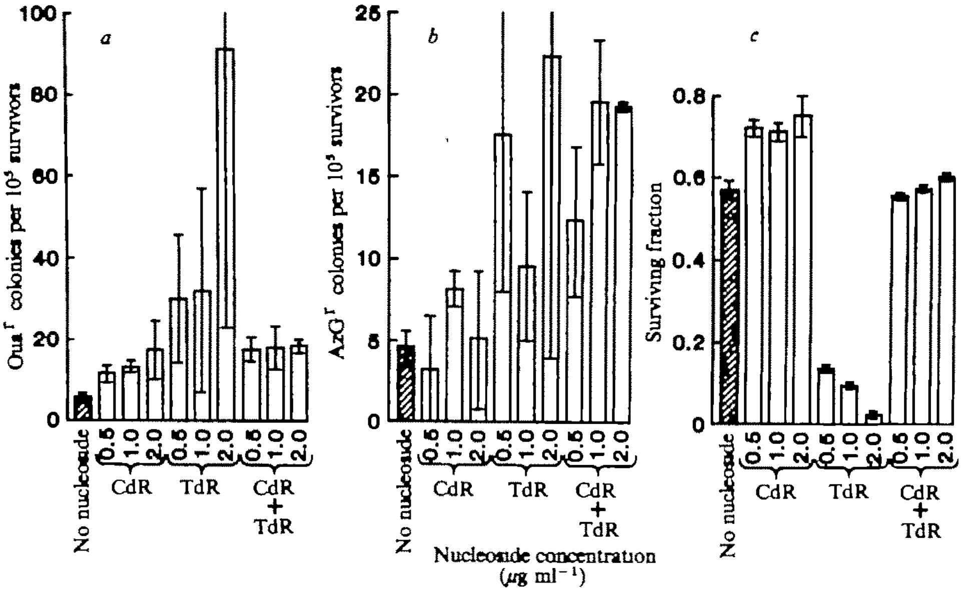

Fig. 1 The induction by MNNG of Oua^r (*a*) and AzG^r colonies (*b*), and the cytotoxicity of MNNG (*c*), in the presence of CdR and TdR in cultures of V79 Chinese hamster cells. For one assay of the induction of AzG^r and Oua^r colonies, 10^5 cells were plated in each of two 100-mm plastic dishes (Corning) in medium of the same composition as used for the cytotoxicity assays. After incubation for 6 h, the cells were treated with 1.0 μg ml^{-1} of MNNG for 2 h, incubated for 48 h in MNNG-free medium containing the indicated supplements of CdR and TdR, trypsinised, replated in fresh medium of the same composition at a density of 0 5–1×10^5 cells per plate in medium containing appropriate supplements, incubated for 24 h, and treated with AzG (40 μg ml^{-1}, 0.26 mM) or with ouabain (3 mM). The cultures were incbubated for 10–12 d with a change of fresh AzG- or ouabain-containing medium supplemented with the indicated levels of CdR and TdR at 5 d. Colonies were fixed, stained and scored 8–10 d later using the same procedures as those used in the cytotoxicity assay. For one assay of cytotoxicity, 200 cells were plated in each of four 55-mm plastic dishes (Corning) in Dulbecco's medium supplemented with 10% dialysed fetal calf serum (DS) (Gibco), or in DS plus various concentrations of TdR and CdR The dishes were incubated for 6 h at 37 °C in a humidified atmosphere of 10% CO_2 in air. Some dishes received 1.0 μg ml^{-1} of MNNG from a stock solution in acetone, others, controls, received 0 25% acetone. After a 2-h incubation, the medium was aspirated and replaced with fresh medium free of MNNG, but containing the supplements of CdR and TdR The cultured were then incubated for 7–9 d, fixed, stained with Giemsa, and the colonies were counted. Only colonies with diameters of 0 5 mm or more were scored using a Biotran automatic colony counter (New Brunswick Scientific). Mutation frequencies (*F*) were calculated by the formula:

$$F = \text{total mutant colonies}/(\text{total cells replated} \times \text{plating efficiency of replated cells}),$$

and also by applying Poisson statistics as described in ref. 29. Both methods of calculation gave essentially the same results The results shown here are the means and ranges of two assays.

yield of Oua^r and AzG^r colonies was reproducibly three- to four-fold greater than with MNNG alone (Fig. 1*a*, *b*).

It is well established that exogenous TdR and CdR, supplied to cell cultures in concentrations of the order of 10^{-5} M (~2.0 μg ml^{-1}) produce an imbalance of the deoxyribonucleotide pools in many lines of mammalian cells, including Chinese hamster ovary cells[17,18]. Moreover, recent studies in cell-free systems show that the fidelity of DNA replication decreases as the relative concentration of incorrect nucleotides increases in the reaction mixture[19]. Therefore, we offer the following interpretation of our data: in V79 cells the imbalance produced in nucleotide pools by the uptake of exogenous TdR and CdR facilitates aberrant replication of DNA alkylated by MNNG, ENNG and EMS. Some errors are lethal, others are mutagenic. The lethal errors are facilitated by TdR, but not by CdR; the mutagenic errors are facilitated by both TdR and CdR.

Our interpretation seems at first to be incompatible with the fact that living systems possess vital 'proof-reading' mechanisms which ensure high fidelity of DNA replication[6]. However, it can be argued that mechanisms which promote infidelity in response to life-threatening environments will be advantageous to living organisms. Radman[20] has proposed such an 'S.O.S.' mechanism; it is considered to operate by mutagenic DNA repair induced in response to genetic damage.

Evidence for a mechanism that need not involve DNA repair, for producing infidelity in DNA replication, is provided by our data (Fig. 1), and by experiments showing increased mutation in Chinese hamster cells grown in medium containing high (4×10^{-3} M) concentrations of thymidine[21], and thymine-starved bacteria[22,23], and yeast[24], which show that DNA synthesis is increasingly error prone when the pools of DNA precursors become unbalanced.

DNA is not the only macromolecule whose synthesis can be made error prone by unbalancing its precursor pools. Starvation of amino acids leads to severe translational errors in bacterial and animal cells[25]. Bujard and C.H.[26] found that *in vitro* transcription of synthetic DNA templates by *Escherichia coli* RNA polymerase became error prone in the presence of 4×10^{-4} M uridine triphosphate, which by nearest-neighbour analysis was shown to be incorporated into RNA in place of cytidine triphosphate. Thus, not only the synthesis of DNA, but also those of RNA and protein can be error-prone in the presence of unbalanced precursor pools, and we propose that such errors could become manifested as substantial alterations in gene expression. The accumulation of copying errors in macromolecule synthesis has been suggested as a mechanism for ageing[27], and has been implicated in mutagenesis and carcinogenesis[15,28]. We consider it likely that errors in DNA, RNA and protein synthesis, facilitated by imbalances in precursor pools, are not only involved in carcinogenesis, mutagenesis and ageing, but have also played a part in the evolution of species.

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Transformed cell lines susceptible or resistant to *in vivo* surveillance against tumorigenesis

THE transformation of a normal cell to one which grows as a cancer requires several steps, one of which may involve the escape from tumour surveillance[1]. We describe here an experiment designed to define these steps more clearly. The principle is to transform normal continuous cell lines *in vitro* and then select for transformants by their growth in agarose[2,3]. These procedures avoid any host selection. By testing the tumorigenicity of these cloned, transformed cell lines in normal and immunodeficient mice, it would be possible to estimate the frequency of transformation resulting in tumorigenic clones and the proportion of these clones that is rejected by normal mice. Based on their tumorigenic potential, we have divided these cells into three classes (Table 1), 0, I, and C. 0 cells, although able to grow in agarose, do not form tumours in either test animal. I cells form tumours only in immunodeficient mice and C cells form tumours in both types of mice. The existence of 0 and I cells indicates that at least one additional mutation is required for these cells to grow as a cancer (C).

Table 1 Classification of cells by growth in agarose and mice

Type	Colony formation in agarose	Growth as tumour in Immune deprived mice	Growth as tumour in Normal mice
N	−	−	−
0	+	−	−
I	+	+	−
C	+	+	+

N cells were grown in Dulbecco's modified Eagle's minimum essential media (DME) plus 10% fetal bovine serum (FBS). These cells were treated with either virus (SV-40 or Abelson murine leukaemia virus) or chemicals (20-methylcholanthrene; *N*-methyl-*N*-nitro-*N*-nitrosoguanidine; 11,12-dihydro-3-methylcholanthrene-11,12-epoxide; or 5,6-dihydrobenz(*a*)anthracene-5,6-epoxide). 24 h later these cells were plated in 0.3% agarose containing DME plus 25% FBS at 10^5, 10^4 and 10^3 cells per 60-mm plate. The plates were incubated at 37 °C until macroscopic colonies appeared, but no longer than 8–12 weeks. Colonies were picked from the agar and grown in DME plus 10% FBS. 5 or 10×10^6 cells were injected subcutaneously into normal mice and mice which had been thymectomised as adults, lethally irradiated (750 rads) and restored with 10^7 syngeneic fetal liver cells (AT×FL).

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Table 2 Distribution of transformed clones characterised by inoculation into normal and AT×FL mice

Transforming agent	Classification of transformed clones			Efficiency of rejection*
	0	I	C	
Viral	14% (2/14)	64% (9/14)	21% (3/14)	75% (9/12)
Chemical	10% (2/21)	19% (4/21)	71% (15/21)	21% (4/19)

The distribution of chemically transformed clones into the 0, I or C categories differs significantly ($P \leq 0.005$)[7] from the distribution of the virally transformed clones.

* Efficiency of rejection = no. of I clones/no. of I+C clones.

Cloned, continuous lines of normal fibroblast cells (N cells) were derived from BALB/c, BALB.B and C57BL/6 embryo cultures. These cells do not form colonies in agarose or grow as tumours even in immunodeficient mice (thymectomy, lethal irradiation and fetal liver restoration of adults; AT×FL). N cells were transformed with either viral or chemical carcinogens (Table 1) and colony formation in agarose was used to select for transformed variants.

As shown in Table 2, 35 independently derived transformed clones were tested for their tumorigenicity in syngeneic normal and immunodeficient mice. Of the 35 cell lines, 14 were derived following viral transformation and 21 were obtained after chemical carcinogenesis. Overall, 31 of 35 clones isolated from agarose formed tumours in AT×FL mice (that is, I+C lines), indicating a high correlation (89%) between *in vitro* growth in agarose and *in vivo* tumorigenicity. The 10–14% of transformed clones which were classified as 0 may represent a class of cells which are either inherently nontumorigenic or which are susceptible to surveillance mechanisms remaining in AT×FL mice. The collection of 0 lines is now being tested for tumorigenicity in mice which have been rendered deficient in other possible surveillance mechanisms.

From the data shown in Table 2, we can calculate that the proportion of tumorigenic transformed cell lines falling into the I-cell category was 9/12 (75%) for the virally transformed lines and 4/19 (21%) for the chemically transformed lines. Expressed in terms of the efficiency of surveillance, 75% of the viral and 21% of the chemically transformed cell lines were susceptible to a surveillance mechanism which is impaired in the AT×FL mouse. Although the AT×FL mouse is severely depleted of T-cell activity, and therefore, immunodeficient[4–6], these animals also have lower levels of natural killer (NK) cell activity. It is tempting to speculate that these deficiencies allow the growth of I lines.

We have not confirmed that all C lines are derived from I-type intermediates as predicted in the N → I → C sequence required by surveillance models. However, it is clear that clones which have undergone the mutation N → I require another mutation, I → C, to grow as a tumour in normal mice. We have several examples where this has occurred.

There are three potential sources of error in categorising I lines. First, the 0 lines could, in fact, be I lines if the AT×FL mouse had residual tumour resistance. Second, if N lines had acquired rejection antigens before transformation, these antigens could result in overestimation of the frequency of I lines; however, the high frequency of C lines makes this unlikely. Third, the transformed clones were tested at 10^7 cells per animal for classification of tumour potential. If surveillance against tumours is of any physiological importance, the system must be capable of eliminating tumour cells when they are in relatively low numbers (certainly less than the test dose of 10^7). At the test dose, efficiency of rejection could have only been underestimated.

To our knowledge, this is the only experiment in which the frequency of appearance of the postulated N → I step has been evaluated by the isolation of transformed clones in the absence of an immune system (that is, *in vitro*) and by testing them simultaneously in immunodeficient (AT×FL) and normal mice. The existence of I cells shows that a host protective mechanism is operative. Although the difference between viral and chemical carcinogenesis seems large, the above sources of error would lead to an underestimation of the efficiency of this host protective mechanism. The resolution of these limitations may only decrease the difference. Consequently, we conclude that immune protective mechanisms have a significant but not absolute role in tumorigenesis.

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Influence of cyclic AMP on intracellular *p*H regulation and chloride fluxes in barnacle muscle fibres

THE *p*H sensitivity of numerous intracellular processes requires the close regulation of internal *p*H (pH_i). As the pH_i of many cells is maintained at a higher value than it would have if H^+ were at equilibrium across the cell membrane, it follows that the acidifying effects of cellular metabolism and of passive H^+ influx and HCO_3^- efflux must be balanced by active acid extrusion. It is now generally accepted that this extrusion is stimulated by decreases in pH_i, and that, in several cells, it is accomplished by exchanging external HCO_3^- for internal Cl^- (refs 1–4). Recently, a role for cyclic 3′,5′-adenosine monophosphate (cyclic AMP) in pH_i regulation was suggested by the observation that dibutyryl cyclic AMP attenuates the CO_2-induced fall of pH_i in mammalian cardiac muscle[5]. We now report experiments on the effects of cyclic AMP on acid extrusion and on accompanying Cl^- fluxes in the barnacle muscle fibre, a preparation known to contain cyclic AMP (ref. 6). Our data show that internally applied cyclic AMP enhances the response of the pH_i-regulating system to acid loads.

The experiments were carried out on superfused single muscle fibres of the giant barnacle (*Balanus nubilus*). Intracellular *p*H and concentrations of low molecular weight solutes were controlled by insertion of a porous dialysis capillary through the fibre, and perfusion with fluid[7]. In one series of experiments, pH_i was monitored by placing *p*H-sensitive and reference microelectrodes into the fibre alongside the dialysis tube. In another series, either Cl^- influx or efflux was measured with ^{36}Cl.

The influence of cyclic AMP on the rate of acid extrusion was examined as shown in Fig. 1*A*. The fibre was loaded with acid by dialysing with a cyclic AMP-free solution at *p*H 6.0 (Fig. 1*A*, segment *ab*); to reduce acid extrusion rate while acid loading, external *p*H (pH_o) was simultaneously lowered from 7.8 to 6.8 (ref. 8). When pH_i had fallen to 7.02 (Fig. 1*A*, point *b*), dialysis was halted so that acid no longer entered the cell by this route, and pH_o was returned to 7.8. In the absence of cyclic AMP, pH_i recovered at a low rate (Fig. 1*A*, segment *bc*). However, when the fibre was dialysed with an acid solution containing cyclic AMP (Fig. 1*A*, *cd*), pH_i recovered nearly four times faster (Fig. 1*A*, *de*). This recovery was blocked (Fig. 1*A*, *fg*) by SITS (4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonic acid), a potent inhibitor of acid extrusion[1,4,9]. Figure 1*B* shows the effect of cyclic AMP on the dependence of acid extrusion rate on pH_i. Although cyclic AMP had little effect at the normal pH_i of 7.3, it greatly increased acid extrusion rate at low pH_i. In these experiments, a high (20 μM) dialysate level of cyclic AMP was used to maintain adequate cytoplasmic levels after dialysis was halted. Even so, the stimulatory effects of the nucleotide faded within 10–20 min unless 3-isobutyl-1-methylxanthine, an inhibitor of cyclic AMP hydrolysis, was added. However, even 1 μM cyclic AMP was able to increase acid extrusion rate when dialysis was continuous, as shown in Fig. 1*C*. Here, dialysis with fluid at *p*H 6.6 caused pH_i to fall (Fig. 1*C*, *ab*) until the entrance of acid by dialysis was balanced by acid extrusion (Fig. 1*C*, *b*). When 1 μM cyclic AMP was now added to the dialysate, pH_i rose by 0.1 (Fig. 1*C*, *bc*), reflecting stimulation of the acid extrusion rate. Exposure to the diuretic furosemide (0.6 mM), an inhibitor of anion transport[10], caused a decline in pH_i (Fig. 1*C*, *cd*), indicating inhibition of acid extrusion.

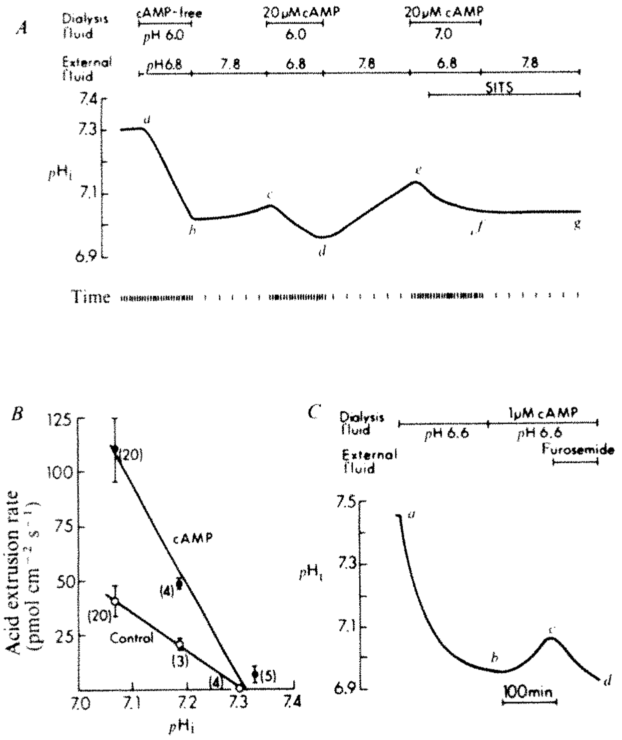

Fig. 1 *A*, Effect of cyclic AMP (cAMP) on acid extrusion. The pH_i was lowered by dialysing, first with cyclic AMP-free (segment *ab*) and then cyclic AMP-containing (*cd*, *ef*) fluid (50 mM HEPES buffer). On halting dialysis, the subsequent recovery of pH_i, which reflects acid extrusion, was slow in the absence of cyclic AMP (*bc*), but much faster in its presence (*de*). SITS (0.5 mM) blocked recovery, even with cyclic AMP (*fg*). Membrane potential (V_m) was −49 mV at the onset of the experiment and −48 mV at the end. The speed of the strip-chart recorder was increased fivefold during the periods of pH_i recovery; each hash mark represents 2.5 min. *B*, Effect of cyclic AMP on the acid extrusion rate at various values of pH_i. Data were derived frpm experiments similar to that above. Values of the acid extrusion rate were calculated from observed d*pH*/d*t* (during pH_i recovery) and measured intracellular buffering power, assuming that the fibre is a cylinder. Vertical bars indicate one standard error; number of experiments given in parentheses. Lines through points represent least-square fits. *C*, Effect of cyclic AMP on pH_i in a continuously dialysed fibre (pH_o = 7.8). During dialysis, pH_i is determined by the balance between introduction of acid by dialysate (*p*H 6.6, 50 mM PIPES buffer) and acid extrusion by the pH_i-regulating system of the cell. Addition of 1 μM cyclic AMP to the dialysate causes a 0.1 increase in steady-state pH_i (compare *b* and *c*), reflecting a stimulation of acid extrusion, which is blocked by the addition of 0.6 mM furosemide (Hoechst) to the external fluid (*cd*). V_m = −48 mV. Unless otherwise indicated, artificial sea water contained (in mM): 418 NaCl, 10 KCl, 11 $CaCl_2$, 32 $MgCl_2$ and 30 HEPES, and was gassed with 100% O_2. Although this solution was nominally HCO_3^--free, $[HCO_3^-]_o$ in the vicinity of the muscle fibre was probably ~1 mM (W.F.B., McCormick and A.R., unpublished). Dialysis fluid contained (in mM): 30 KCl, 160 K glutamate, 20 Na glutamate, 4 $MgSO_4$, 550 mannitol, 0.5 EGTA, 0.5 phenol red and 50 mM HEPES or PIPES. Temperature was 22 °C.

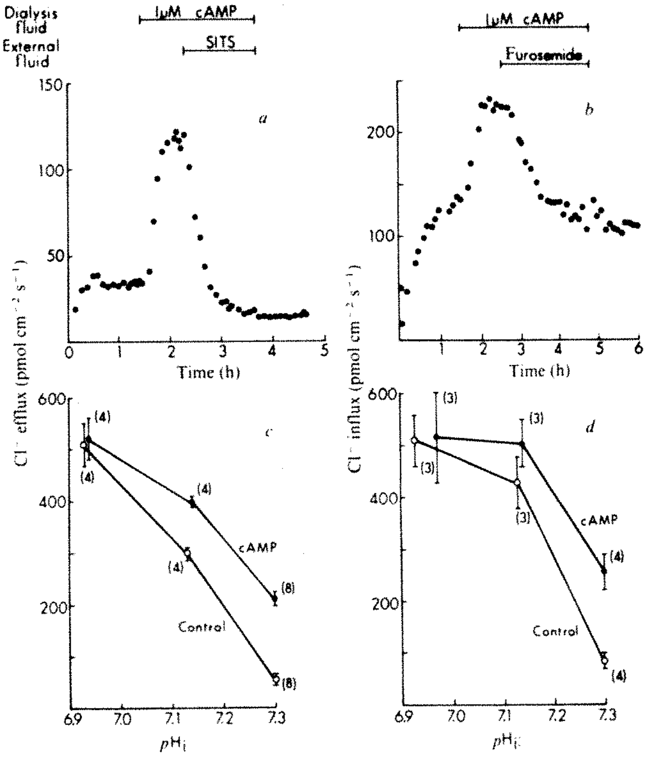

Fig. 2 *a*, Effect of cyclic AMP on Cl^- efflux. At time zero, flow of isotope-containing dialysis fluid, *p*H 7.3 (50 mM HEPES buffer) was begun. After Cl^- efflux had reached a steady state, 1 μM cyclic AMP was added to the dialysis fluid, causing an increase in Cl^- efflux. Subsequent addition of 0.5 mM SITS to the external fluid reduced Cl^- efflux to slightly below control values. V_m was −53 mV at the outset and −49 mV at the end of the experiment. *b*, Effect of cyclic AMP on Cl^- influx. After Cl^- influx had reached a steady state, addition of 1 μM cyclic AMP to the dialysis fluid caused an increase in Cl^- influx which was then reversed by the presence of 0.6 mM furosemide in the external fluid. V_m was −51 mV and −47 mV at the beginning and end of the experiment, respectively. *c*, *d*, Effect of cyclic AMP on Cl^- efflux and influx at various values of pH_i. Experiments were similar to those above. Vertical bars represent one standard error; number of experiments is given in parentheses. Lines through points drawn by hand. Actual values of pH_i were verified with *p*H microelectrodes (see Fig. 1*C*) in separate experiments; 4–5 determinations of pH_i were made for each *p*H of the dialysis fluid; s.e. = ±0.03–0.04.

Evidence that acid extrusion involves an exchange of external HCO_3^- for internal Cl^- (refs 1, 2), and the effect of cyclic AMP on acid extrusion, suggested that cyclic AMP would stimulate Cl^- efflux. Figure 2*a* and *b* shows that dialysis with 1 μM cyclic AMP stimulated not only Cl^- efflux, but also its influx. These effects could be blocked by either SITS or furosemide. Lowering pH_i stimulated efflux and influx (as it did acid extrusion rate),

Table 1 Dependence of Cl^- influx and rate of acid extrusion on $[HCO_3^-]_o$ at different values of pH_i

pH_i	Cl^- influx (pmol cm^{-2} s^{-1})		Acid extrusion rate (pmol cm^{-2} s^{-1})	
	$[HCO_3^-]_o$ = 0	$[HCO_3^-]_o$ = 6 mM	$[HCO_3^-]_o$ = 0	$[HCO_3^-]_o$ = 6 mM
7.30	51±2 (3)	48±1 (3)	0	12±8 (6)
7.13	455±44 (3)	310±23 (3)	32±8 (4)	171±32 (6)
6.93	512±46 (3)	351±44 (3)	101±18 (4)	436±110 (3)

External solutions (pH_0 = 7.8) contained either nominally 0 mM HCO_3^- (gassed with 100% O_2) or 6 mM HCO_3^- (gassed with 0.4% CO_2, balance O_2); all solutions contained 30 mM HEPES. In acid extrusion experiments, fibres were acid loaded using the NH_4Cl prepulse technique[8]. The values are means ± s.e.; numbers of experiments in parentheses.

and these responses were further enhanced by cyclic AMP (Fig. 2*c*, *d*). Cyclic GMP did not significantly alter Cl^- efflux or influx, or the acid extrusion rate.

At pH_i 7.3, cyclic AMP stimulated both unidirectional Cl^- fluxes (Fig. 2*c*, *d*), whereas it had little effect on acid extrusion (Fig. 1*B*), that is, net HCO_3^- influx. As these increases in Cl^- efflux and influx are of similar size (~160 pmol cm^{-2} s^{-1}), it is likely that, at pH_i 7.3, cyclic AMP enhances a Cl–Cl exchange. The existence of a Cl–Cl exchange at pH_i 7.3 is supported by the requirement of Cl^- influx for internal Cl^- (J.M.R. and M.S.B., unpublished), and by our observation that, with or without cyclic AMP, Cl^- efflux and influx were inhibited to a similar extent by either SITS or furosemide. Our data indicate that reducing pH_i below 7.3 enhances an existing Cl–Cl exchange (Fig. 2*c*, *d*) and, in addition, initiates a HCO_3–Cl exchange, that is, acid extrusion (Fig. 1*B*). Both these exchanges were stimulated by cyclic AMP. The common sensitivity, at low pH_i, of the Cl–Cl and HCO_3–Cl exchanges to cyclic AMP, SITS and furosemide suggests that both exchanges are mediated by the same carrier. If this is the case, then HCO_3^- and Cl^- might compete for entry. This expectation is confirmed in Table 1. At pH_i 7.3, when the acid extrusion rate was zero and the exchange mechanism was in the Cl–Cl mode, increasing $[HCO_3^-]_o$ had little effect on either acid extrusion rate or Cl^- influx. However, at lower values of pH_i, raising $[HCO_3^-]_o$ not only increased the acid extrusion rate, but also decreased Cl^- influx. We suggest that pH_i regulation in barnacle muscle is accomplished by an anion exchange mechanism whose rate is enhanced by cyclic AMP and by decreases in pH_i. At pH_i 7.3, this exchange operates at a low rate in the Cl–Cl mode. At lower pH_i, the rate of exchange increases, and simultaneously the mode of exchange shifts from Cl–Cl to HCO_3–Cl. Cyclic AMP increases the rate of anion exchange in either mode, thereby promoting the fluxes of both HCO_3^- and Cl^-.

Our observations concerning the effects of cyclic AMP on pH_i regulation may have implications for any cell subjected to acid loads. Mammalian cells are of particular interest because their high metabolic rate and presumed large HCO_3^- efflux represent a sustained acid load, comparable with that in the acid-dialysed barnacle muscle fibre of Fig. 1*C*. Thus, hormones which increase cyclic AMP levels might raise pH_i, and thereby affect a variety of processes, with the pH_i changes serving as a third messenger.

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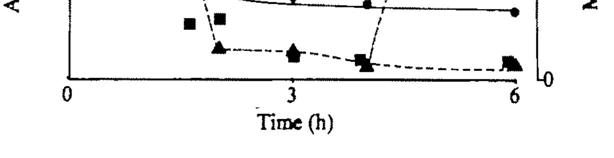
Influence of ATP and calcium on the cortical reaction in sea urchin eggs

THE prevention of polyspermy in the sea urchin egg is a two-stage process: a rapid reduction in the chances of refertilisation—the 'fast block'[1,2], which seems to be electrical in nature[3]—and a slower block that coincides with elevation of a morphological barrier, the fertilisation membrane. The fast block has many features in common with the action potential of excitable tissues, while elevation of the fertilisation membrane seems to involve a classical exocytotic reaction similar to that found at nerve terminals. Lying immediately beneath the plasma membrane of unfertilised eggs is an array of vesicles called cortical granules each about 1 μm in diameter. Shortly after fertilisation the contents of these granules are expelled, the granule membrane becoming incorporated into the plasma membrane of the egg. The trigger for initiating the cortical reaction seems to be a rise in intracellular ionised calcium. Thus granule release can also be promoted by the ionophore A23187 (ref. 4) and measurements in aequorin-loaded fish and sea urchin eggs[5,6] show that exocytosis brought about by both sperm

Fig. 1 Influence of cyanide on the cortical reaction and level of ATP in eggs of *Echinus esculentus* at 16 °C. ●, ○ ATP content of eggs treated at zero time with 5 mM cyanide in artificial seawater containing (mM) NaCl, 465; $MgCl_2$, 55; $CaCl_2$, 11; KCl, 10; $NaHCO_3$, 2.5 pH 7.8. ATP was assayed fluorimetrically[8]. Fertilisation membrane elevation in response to sperm (▲, △) and the ionophore A23187 (20 μg ml^{-1}) (■, □) is also shown. After 4 h cyanide was removed from an aliquot of poisoned eggs by thorough washing (○, △, □).



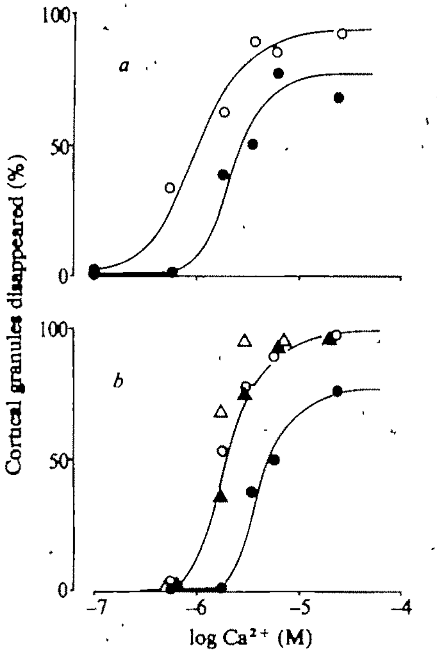


Fig. 2 Dependence of the cortical reaction in broken eggs of *Echinus esculentus* on ionised calcium at 16 °C. *a*, Eggs subjected to a high voltage discharge (1 kV cm^{-1}, $\tau = 50$ μs)[10] in a medium containing (mM): potassium glutamate, 250, glycine, 500, NaCl, 10; $MgCl_2$, 10; HEPES 10, *p*H 6.7. The ionised calcium concentration was controlled with 10 mM EGTA, the abscissa representing values calculated from the stability constants[11]. Thirty seconds after shocking, the eggs were fixed with 4% formaldehyde[2] and the number of cortical granules scored by high-power phase contrast microscopy. ○, Control eggs; ●, eggs treated for 4 h with 5 mM cyanide. *b*, eggs attached to glass or plastic with poly-L-lysine (1 mg ml^{-1}) and broken in the artificial ooplasm described in (*a*). The portion of egg cortex left on the support could be scored at 30 s for the number of cortical granules reacted. ○, Control eggs; ●, cyanide-poisoned eggs. Some eggs were broken in an artificial ooplasm containing 10 mM ATP: △, Control eggs; ▲, cyanide-poisoned eggs. Each point represents a separate experiment on cortices prepared from the same batch of eggs. The reproducibility of individual observations was better than ±10%.

and ionophore is associated with a rise in intracellular ionised calcium. The mechanism of exocytosis in any system is unknown and the cortical granule reaction in sea urchin eggs seems to provide an excellent experimental preparation for its investigation. Here we examine the influence of metabolic poisons and calcium on the cortical reaction. In the presence of ATP exocytosis is activated by micromolar concentrations of calcium; but in the nominal absence of ATP the exocytotic process slowly becomes refractory to calcium.

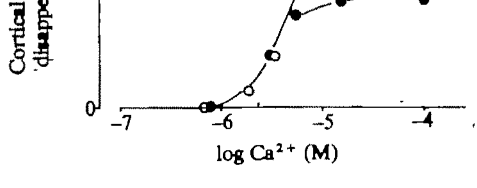
Eggs of *Echinus esculentus* were used and the methodology was as described previously[2]. Figure 1 shows results obtained with intact eggs. The metabolic poison cyanide reversibly blocks cortical granule release and fertilisation membrane elevation in response to both sperm and ionophore. These effects do not result from an action of cyanide *per se* on the activating agent because control eggs elevate normal membranes when exposed to sperm or ionophore in cyanide. Essentially similar results have been obtained with dinitrophenol (DNP) (0.2 mM, *p*H 7.0)[7]. Also, the onset of inhibition of cortical granule release closely parallels a fall in energy-rich phosphate compounds in the egg.

These observations on poisoned eggs can be explained in a number of ways—for example, poisoning may deplete the cell of something essential for activation, or increase the level of an inhibitor. To investigate these possibilities we examined the cortical reaction under controlled conditions using two partially broken egg preparations. In the first, rapid access to the interior of the cell was obtained by exposing suspensions of eggs to a high-voltage discharge[9,10]. Eggs were suspended in a medium similar to ooplasm. If Ca^{2+} is present in the medium at the time the shock is delivered, unpoisoned eggs subsequently undergo cortical granule release and raise a fertilisation membrane; but this response is not seen in EGTA. This technique is a modern version applicable to populations of cells of the 'pricking' technique used by many early workers to activate eggs. Figure 2 shows that the buffered Ca^{2+} concentration required to effect the release of half the cortical granules in unpoisoned eggs is close to 1 μM. This value is more than an order of magnitude lower than the only other reported measurement[6]. Also shown are the results of applying the high-voltage technique to fully-poisoned eggs. Rather surprisingly both cortical granule release and fertilisaton membrane elevation can be fully activated; but the concentration of Ca^{2+} required to effect the release of half the granules, 3 μM, is somewhat higher than in unpoisoned eggs.

Another broken cell preparation has been described by Vacquier[12]. Eggs are attached to a surface by protamine sulphate and subjected to a shearing force. The eggs break leaving small portions of egg cortex attached to the surface. These portions of cortex respond to Ca^{2+}; but are reported to undergo granule–granule fusion. We have found that if the technique is modified by attaching jelly-free eggs either directly to plastic or to a surface coated with poly-L-lysine[13] and the eggs broken in the medium described above, the resulting portions of egg cortex are activated in the same range of Ca^{2+} concentrations as eggs broken by shocking and the appearance of the reacted cortices is entirely consistent with exocytosis, that is, granule–plasma membrane fusion. Isolated cortices prepared from cyanide-poisoned eggs resemble 'shocked' eggs in that they need a higher Ca^{2+} concentration to activate cortical granule discharge. Addition of ATP (10 mM) to these cortices without a change in *p*H restores a Ca^{2+}-sensitivity close to that of unpoisoned eggs. Figure 2 also shows that inclusion of this concentration of ATP in the medium used to prepare cortices from unpoisoned eggs gives the highest apparent affinity of the cortical response for calcium which is very similar to that found in 'shocked' eggs.

The difference in apparent affinity between eggs subjected to a high-voltage discharge (Fig. 2*a*) and eggs broken (Fig. 2*b*) in Ca^{2+}–EGTA solutions containing no ATP emphasises the dependence of the Ca^{2+}-affinity of the cortical reaction on the levels of nucleotide. The high-voltage technique allows rapid access of the Ca^{2+} buffers into the egg, while maintaining sufficient integrity to retain some ATP and ATP-regenerating systems. Breaking the egg removes the cortex from the remainder of the egg and one would expect the local ATP concentration to be much lower as a consequence. Despite the lower Ca^{2+}-affinity in eggs broken in the absence of ATP, the cortical reaction is comparable to that of unpoisoned 'shocked' eggs in

Fig. 3 Dependence of cortical granule exocytosis on ATP at 14 °C. Portions of cortex stuck to glass coverslips with poly-L-lysine and isolated in a nominally Ca^{2+}-free medium containing 1 mM EGTA and 5 mM ATP were superfused with ATP-free medium for 10 min (○) or 30 min (●) before exposure to calcium. The ordinate is the proportion of cortical granules that had disappeared 30 s after the solution had been changed to one containing calcium.



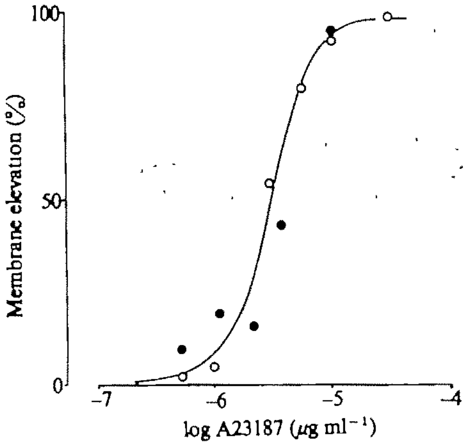


Fig. 4 Concentration–response curve for the ionophore A 23187 in ASW (○), and in Ca^{2+}-free ASW containing 5 mM EGTA (●) at 16 °C. Membrane elevation was scored 3 min after addition of ionophore.

that it proceeds to completion. This contrasts with the cortical reaction of cyanide-poisoned eggs where, in addition to a shift in Ca^{2+}-affinity, some other change seems to have taken place because relatively high (100 μM) levels of Ca^{2+} are unable to effect the release of a proportion (about 15%) of the cortical granules. This effect can be mimicked in isolated cortices by superfusing for various times with nominally ATP-free solutions containing a buffered ionised calcium concentration of less than 10^{-7} M. Subsequent readmission of Ca^{2+} after exposure to ATP-free media for 10 and 30 min triggers appreciable exocytosis (Fig. 3). It is, however, noticeable that although the apparent affinity of exocytosis for Ca^{2+} remains constant, the fraction of granules released falls progressively and the unreleased granules are refractory to very high (0.5 mM) concentrations of calcium. Control cortices exposed for 30 min to Ca^{2+}-free media containing ATP subsequently respond normally to calcium. These results imply that a supply of free ATP is not necessary for exocytosis in this preparation; but the progressively increasing number of cortical granules that fail to respond to Ca^{2+} after prolonged exposure to ATP-free media suggests that tightly-bound ATP, or something derived from it, is necessary either to energise exocytosis or to confer Ca^{2+}-sensitivity on it. After prolonged exposure of isolated cortices to ATP-free media, readmission of ATP restores the high affinity of the exocytotic reaction for Ca^{2+} but fails to restore Ca^{2+}-sensitivity to the refractory granules.

Although there is a slow onset of refractoriness in the cortical response of poisoned eggs, cortices isolated from eggs in which the cortical reaction to sperm and ionophore is completely blocked still react when challenged with quite low concentrations of calcium. This is a rather surprising observation because ionophore, in particular, might be expected to promote Ca^{2+} entry across the plasma membrane until the threshold for exocytosis is reached. That this may not be so is suggested by the data in Fig. 4 which show that there is virtually no difference in the ability of ionophore to activate unpoisoned eggs in normal and Ca^{2+}-free seawater containing EGTA and measurements of ^{45}Ca^{2+} influx show very little stimulation by ionophore. As measurements with aequorin show that ionophore increases intracellular ionised calcium[5], the source of this Ca^{2+} must be intracellular. In seeking an explanation for these results it should not be forgotten that poisoned eggs may contain a potent inhibitor of the cortical reaction, but another attractive possibility is that the cortical reaction in intact eggs involves the release of Ca^{2+} from an internal store. The data here might be explained if this store only remains filled in the presence of ATP. In the absence of this store, ionophore would not be able to generate a sufficient rise in ionised calcium in the vicinity of the cortical granules to initiate exocytosis. This suggestion finds some support in the finding that isolated cortices bind ^{45}Ca^{2+}. This binding is ATP-dependent, is much larger in cortices with intact cortical granules than in cortices that have undergone the cortical reaction, and is much reduced in the presence of the calcium ionophore A 23187.

There is another difference in the response of egg cortices prepared from unpoisoned and poisoned eggs. The response to low concentrations of Ca^{2+} in cortices prepared from unpoisoned eggs often starts at one or a few points—usually near the edge of the torn piece of cortex—and spreads in a wave or waves across the preparation. This is never seen in cortices prepared from poisoned eggs where the cortical granules respond in an apparently random fashion. This propagation reaction seen in unpoisoned cortices may also be related to the existence of a labile calcium store. Thus a rise in ionised calcium at one point may promote release of calcium from the store in a way analogous to calcium-dependent release of calcium in the sarcoplasmic reticulum[14]. Treatment of isolated cortices with ionophore in the presence of EGTA should deplete any membrane-bound stores of calcium and permit examination of cortical granule release in the absence of the presumed store. The cortical reaction of cortices prepared from unpoisoned eggs and treated in this way closely resembles that of cortices prepared from poisoned eggs except that addition of ATP fails to induce the high calcium sensitivity characteristic of unpoisoned eggs. These findings with ionophore-treated cortices support the concept of a store but raise the possibility that the rapid shift in affinity for calcium induced by ATP may be a property of the store and not of the exocytosis reaction itself and suggest that the existence of a store of releasable calcium may be a complicating factor in studies of calcium-dependent exocytosis in this preparation.

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Novel cytochrome *b* system in phagocytic vacuoles of human granulocytes

NEUTROPHIL granulocytes are the most numerous leukocytes and have a primary microbicidal role. They are the first cells to accumulate at sites of inflammation where they engulf, kill and digest microbes. The phagocytic process is accompanied by a burst of oxygen consumption which is important for the killing of certain bacteria, as the ability to kill bacteria is impaired in anaerobic conditions[1]; in chronic granulomatous disease (CGD) this enhancement of oxygen consumption is distinctively lacking[2]. The burst of oxygen metabolism is not inhibited by cyanide

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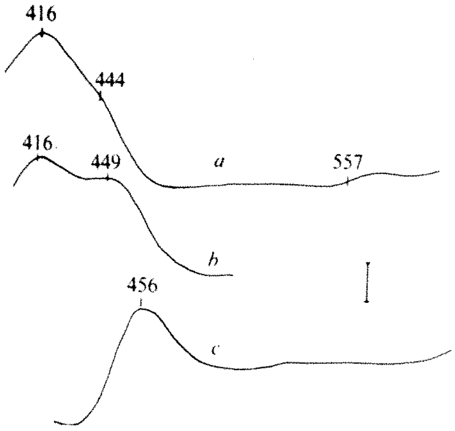


Fig. 1 Difference spectra of human neutrophils incubated with latex particles (*a*), latex particles in the presence of (2 mM) KCN (*b*) or phorbol myristate acetate (*c*). Absorbance scale = 0.028 of full scale for *a* and *b* and 0·011 for *c*. Spectra were determined in the few minutes after mixing 10^8 purified neutrophils[5] in 3 ml of Hanks BSS with 2.5×10^9 latex particles preopsonised with human IgG (ref. 12) or with 7.5 μg of phorbol myristate[13] in 10 μl DMSO.

or azide[3], distinguishing it from mitochondrial respiration by these cells which contain few mitochondria and derive most of their energy from glycolysis. The subcellular localisation and mechanism of this oxidase system have been the subject of considerable investigation and controversy. We describe here a cytochrome *b* system which becomes incorporated into the phagocytic vacuoles of human neutrophils. It is distinct from the cytochrome systems of the endoplasmic reticulum and mitochondria, and abnormalities in patients with CGD implicate it as an essential component of the microbicidal oxidase system.

Human neutrophils were purified by dextran sedimentation of erythrocytes, centrifugation through a gradient of Ficoll–sodium metrizoate and hypotonic lysis of residual erythrocytes. The effect of stimuli that initiate the metabolic burst on the visible light spectrum was examined in a split-beam spectrophotometer[4] after the addition to a suspension of purified neutrophils of latex particles opsonised with IgG or phorbol myristate acetate. This resulted in marked spectrophotometric changes which differed for the two stimuli. The latex particles produced a broad peak at about 416 nm with a shoulder at 442 nm and a minor peak at about 560 nm (Fig. 1). The 416-nm peak was reduced by the addition of KCN (2 mM) revealing the shoulder at 442 nm as a distinct peak at 449 nm. Phorbol myristate produced a peak at 456 nm with a secondary smaller peak at $\sim$430 nm.

The possibility that these spectroscopic changes could be related to phagocytic vacuoles was then examined as this is the site where the oxidase system unites with the engulfed microbe[5]. A diaphorase enzyme system in neutrophils has been located in the plasma membrane, which invaginates to form the walls of the phagocytic vacuole and has been shown to be abnormal in CGD[6]. Phagocytic vacuoles (Fig. 2) were isolated by flotation on sucrose gradients followed by differential centrifugation. The preparation contained latex particles, most of which were enclosed in bilamellar membranes. Granule contents could be clearly seen to have been released into some of the phagosomes. Concanavalin A binds to the surface of neutrophils[7] and a radiolabelled derivative was used as a plasma membrane marker at concentrations well below those that result in metabolic stimulation of the cell[7]. Ten per cent of the radioactive lectin became bound to the cells and 1·44% of the total activity in the homogenate was recovered with the phagosome preparation. This was found to be free of measurable contamination by endoplasmic reticulum as it contained less than 0·004% of the marker enzyme NADPH cytochrome *c* reductase[5] present in the homogenate, and lacked spectrophotometric evidence of cytochrome P450 (ref. 8). Complete lack of spectroscopic evidence of cytochrome oxidase indicated little mitochrondrial contamination[8].

Dithionite difference spectra of these vacuoles gave distinct peaks at 429 and 560 nm at room temperature, characteristic of a cytochrome *b* (ref. 8). These peaks were more clearly defined at slightly shorter wavelengths when examined at 77 K (Fig. 3).

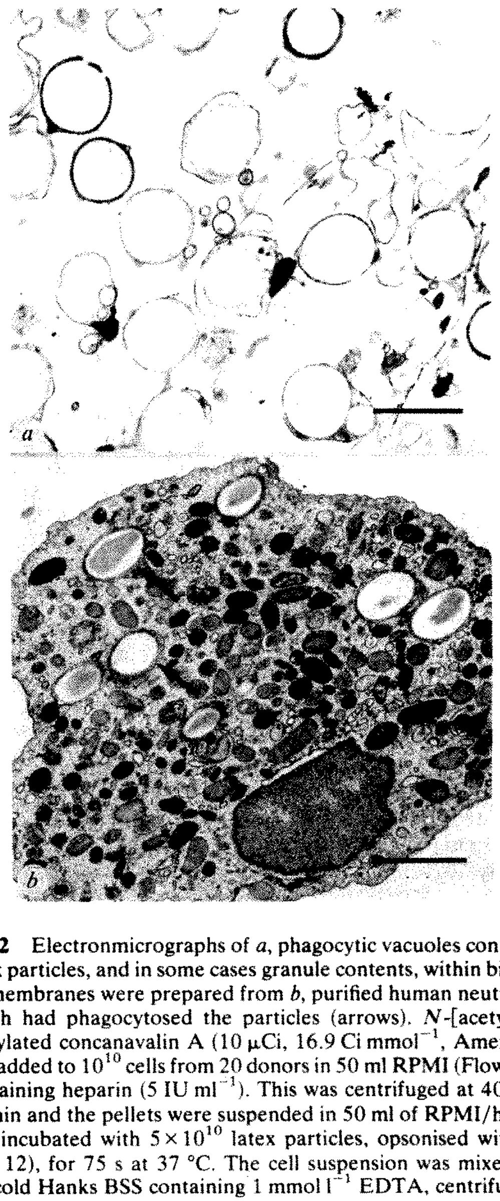


Fig. 2 Electronmicrographs of *a*, phagocytic vacuoles containing latex particles, and in some cases granule contents, within bilamellar membranes were prepared from *b*, purified human neutrophils which had phagocytosed the particles (arrows). *N*-[acetyl-^{3}H]-acetylated concanavalin A (10 μCi, 16.9 Ci mmol^{-1}, Amersham) was added to 10^{10} cells from 20 donors in 50 ml RPMI (Flow Labs) containing heparin (5 IU ml^{-1}). This was centrifuged at 400*g* for 10 min and the pellets were suspended in 50 ml of RPMI/heparin and incubated with 5×10^{10} latex particles, opsonised with IgG (ref. 12), for 75 s at 37 °C. The cell suspension was mixed with ice-cold Hanks BSS containing 1 mmol l^{-1} EDTA, centrifuged at 400*g* for 10 min, and the pellets were homogenised in 15 ml of 0.34 mol l^{-1} sucrose in a Douce homogeniser with 100 strokes of a B pestle. The homogenate was mixed with 30 ml of 50% sucrose and 15 ml of the mixture was placed in the bottom of a 60 ml Beckman centrifuge tube and overlaid with 30 ml of 23% and then 10 ml of 11.2% sucrose and centrifuged at 100,000*g* for 10 h at 4°C in a Beckman L2-65B centrifuge. The material at the interface between the 11.2% and 23% sucrose was aspirated, the sucrose concentration adjusted to 0.34 mol l^{-1} with H_2O containing 1 mM EDTA and 5 IU ml^{-1} heparin, and centrifuged at 27,000*g* for 15 min in a Sorvall SS-3 centrifuge. The pellets were resuspended in 0.34 mol l^{-1} sucrose which, like all the other sucrose solutions, contained 1 mmol^{-1} EDTA and 5 IU ml^{-1} heparin. Scale bars, 1 μm.

A carbon monoxide difference spectrum produced a small peak at 420 nm which could indicate binding of CO by the cytochrome *b*. Such a CO-bonding pigment may function as an oxidase (cytochrome o) of a type which has not been previously described in mammalian cells[8]. Dithionite difference spectra on

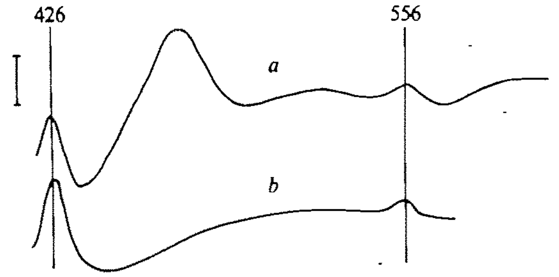
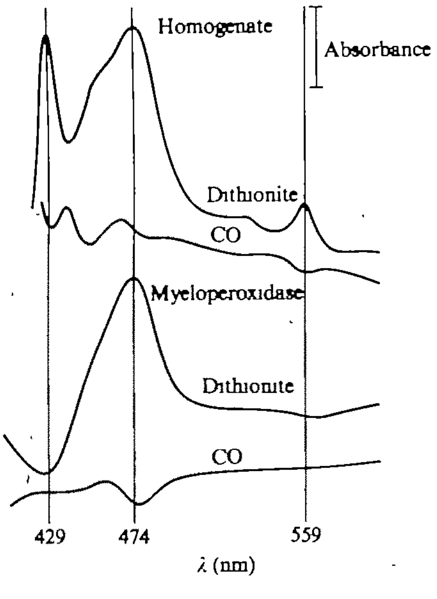


Fig. 3 Dithionite difference spectra at 77 K of a whole neutrophil homogenate (*a*) and a purified preparation of phagocytic vacuoles (*b*) (see legend to Fig. 2 for experimental details). Absorbance scale = 0.0056 and 0.0028 of full scale for *a* and *b* respectively.

the whole homogenate gave a similar pattern with, in addition, a peak at 475 nm and a minor broad peak between 510 and 540 nm. Similar difference spectra were observed in three separate homogenates each containing pooled cells from 20 normal subjects, and in 15 homogenates of cells from individuals. The 475-nm peak was identified as that of myeloperoxidase using purified human material[9] for which the dithionite difference spectra gave a classical peak at 475 nm which was shifted slightly to 462 nm by the addition of CO (Fig. 4). There were no obvious peaks at lower wavelengths. There was no spectroscopic evidence of myeloperoxidase in the phagocytic vacuole preparation (Fig. 3) which was calculated to contain ~2.4% of the cytochrome *b* in the whole homogenate by comparing the integrated areas under the 560-nm peaks.

These studies indicate the presence of a relatively pure cytochrome *b* in the phagocytic vacuoles which is distinct from both the P450 system in the endoplasmic reticulum and the mitochondrial cytochrome system. This cytochrome seems to be a likely component of the microbicidal oxidase system and its spectrum was thus examined in neutrophil homogenates from patients with CGD. This condition is characterised by defective activity of this oxidase system[3] and impaired killing of certain bacteria, leading to recurrent pyogenic infections which are often fatal. The characteristic spectrum of cytochrome *b* was missing or grossly abnormal in cell homogenates in all six of the patients that we have studied[10]. Half the normal level of this cytochrome was found in the two, clinically unaffected, maternal obligate heterozygote carriers[10] of this sex-linked disease[11], indicating that the spectral changes observed in the patients were not simply a consequence of ill health.

Fig. 4 Dithionite and CO difference spectra of an homogenate of neutrophils from a pool of 20 normal donors and of purified human myeloperoxidase. Neutrophils were purified and homogenised in 0.34 mol l^{-1} sucrose as described in Fig. 2, and 4 mg of protein was added to each cuvette. Absorbance scale = 0 0056 of full scale.



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24, 25-Dihydroxyvitamin D is a metabolite of vitamin D essential for bone formation

THE question whether vitamin D acts directly at sites of bone formation or indirectly at sites of calcium and phosphate absorption has long been a source of argument, with no general conclusion reached. Treatment of rachitic chicks with 1-hydroxyvitamin D_3, the synthetic analogue of 1,25-dihydroxyvitamin D_3, does not prevent most of the changes seen in bone affected by rickets, in spite of normal plasma levels of calcium and inorganic phosphate (unpublished). Similar findings have been reported for man[1], suggesting that vitamin D affects bone formation directly and that the effect is mediated by a metabolite(s) other than 1,25-dihydroxyvitamin D_3. In normocalcaemic animals, the predominant dihydroxylated metabolite of vitamin D in the blood is 24, 25-dihydroxyvitamin D and, like 1,25-dihydroxyvitamin D, it is synthesised in the kidney[2,3]; but nothing is known of its function. Improved calcium retention has been reported in anephric patients treated with 24,25-dihydroxyvitamin D_3 (ref. 4), which has been observed to accumulate preferentially in fetal bones of rat[5] and in callus tissue formed after experimental fracture (unpublished). We now

Table 1 Body weight and plasma concentration of calcium and inorganic phosphate of chicks treated with vitamin D derivatives

Treatment	Body weight (g)	Plasma Ca (mg per 100 ml)	Plasma Pi (mg per 100 ml)
Vitamin D_3	264.2±5.3	10.9±0 40	6.8±0 22
24,25-Dihydroxyvitamin D_3	266.7±9.2	9 4±0.45	6.3±0 17
24,25-Dihydroxyvitamin-D_3 + 1 hydroxyvitamin D_3	289.2±7.9	10.4±0 22	6 9±0 31
None	211.7±9.0	6.3±0 12	5.8±0.17

All figures are mean values for six birds ±s.e.m

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Table 2 Comparison of tibial epiphyses, metaphyses and diaphyses in the different groups

24,25-Dihydroxyvitamin D_3	1-Hydroxyvitamin D_3 + 24,25-dihydroxyvitamin D_3	None ($-D_3$)	Vitamin D_3	Treatment
3.3±0.3	3.4±0.3	5.0±0.4	3.5±0.2	Width of cartilage in epiphyses (mm)
25±4	26±4	35±8	28±6	Width of metaphyseal bone trabeculae (μm)
9±2	5±1	16±3	6±1	No. of osteoclasts per mm in the metaphysis
+	+	++	±	Hypertrophied osteocytes in the diaphysis
±	±	+++	±	Connective and preosteogenic tissue between trabeculae in metaphysis
+++	++	+	++	Haemopoietic bone marrow in metaphysis

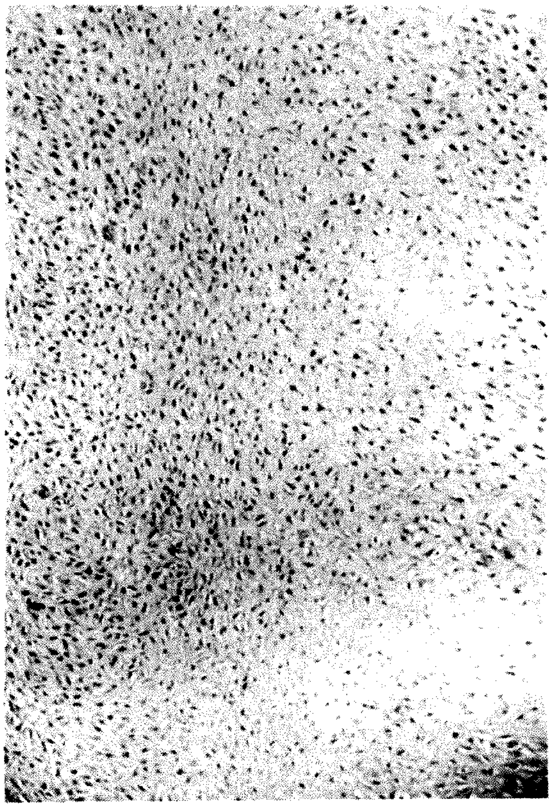
Measurements were made by projecting the haematoxylin and eosin stained para-sagittal longitudinal sections of tibiae on a microscope screen. The width of the epiphyses was measured ×40 and that of metaphyseal trabecule ×400. Metaphyseal osteoclasts were counted for areas 0.5×0.5 mm (×400) and calculated per mm^2 as described before[8]. The figures are mean values±s.e.m. of 5–10 measurements from each chick, six chicks in each group.

report that when rachitic chicks are exposed to various therapeutic treatments with metabolites of vitamin D, the presence of 24,25-dihydroxyvitamin D is essential for the healing process.

One-day-old male chicks (*Gallus domesticus*) were depleted of vitamin D by feeding them a vitamin-D-deficient diet[6] for 4 weeks. They were then divided into four groups and injected subcutaneously daily with, respectively, vitamin D_3; 24,25-dihydroxyvitamin D_3; 24,25-dihydroxyvitamin D_3 and 1-hydroxyvitamin D_3, or nothing but propylene glycol. After 11 d the chicks were weighed and killed, and plasma was prepared for the estimation of calcium and inorganic phosphate as before[7](Table 1). Proximal tibiae were removed, fixed in formalin, decalcified in formic acid and embedded in paraffin. Longitudinal 6–7-μm sections were stained with haematoxylin and eosin or with toluidine blue and examined by light microscopy. The length of the cartilaginous epiphyses, the width of the metaphyseal trabeculae and the number of metaphyseal osteoclasts were compared between groups. Other important morphological findings were graded from ± to +++ (Table 2).

As Table 1 shows, the vitamin-D-deficient chicks, compared with the vitamin-D_3-treated chicks, showed hypocalcaemia ($P<0.01$) and hypophosphataemia ($P<0.01$) associated with growth retardation ($P<0.05$). 24,25 Dihydroxyvitamin-D_3-treated birds grew as well as those treated with vitamin-D_3, but were unable to maintain normal plasma levels of calcium ($P<0.05$). Only birds treated with both 24,25-dihydroxyvitamin D_3 and 1-hydroxyvitamin D_3 maintained normal plasma levels of both calcium and inorganic phosphate, and their growth was apparently better than that of vitamin-D_3-treated birds ($P<0.05$).

Fig. 1 Tibial epiphysis from a vitamin-D-depleted chick. Note typical rickets; there is a wide zone of abnormally orientated chondrocytes between the proliferating zone and the zone of provisional calcification. (Haematoxylin and eosin×120.)



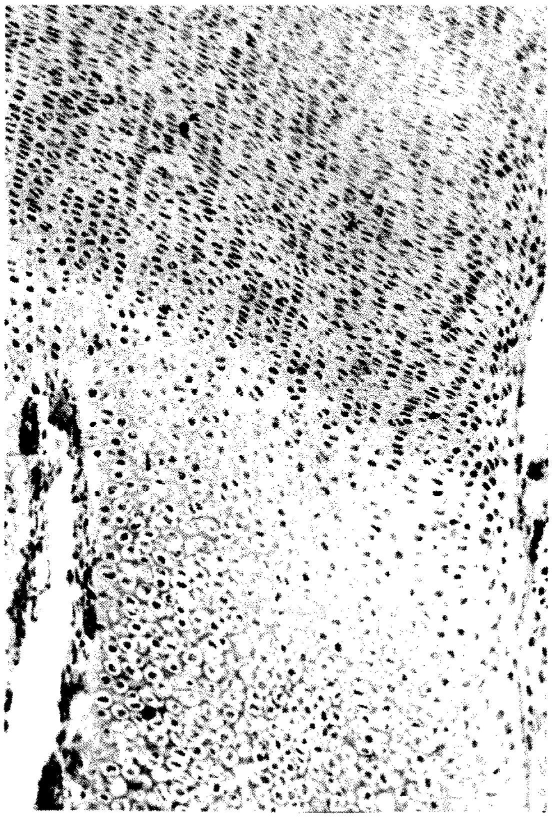


Fig. 2 Tibial epiphysis from a chick treated with 24,25-dihydroxyvitamin D_3 and 1-hydroxyvitamin D_3. Note normal arrangement of the proliferating, maturing and hypertrophic zones. (Haematoxylin and eosin×120.)

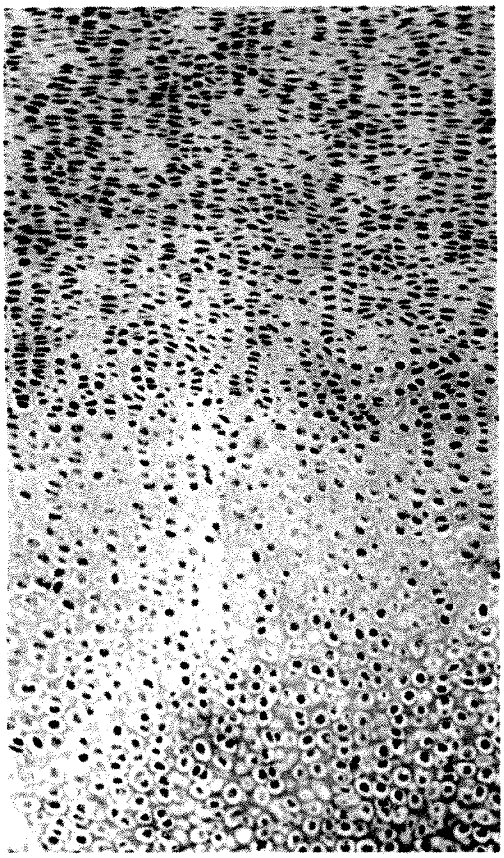
The tibial epiphyses in all birds were still cartilaginous. In the controls (vitamin-D_3-treated birds) the different zones of the epiphyses, which were 3.5±0.2 mm long (Table 2) were well defined. There was a straight demarcation line between the proliferating, maturing and hypertrophic zones. Metaphyseal trabeculae were parallel to the long axis of the tibiae. There were only a few osteoclasts (Table 2).

In the vitamin-D-deficient chicks the tibial epiphyses were longer—5.0±0.4 mm (Table 2). The zone of maturation was wide and disorganised, and the demarcation line between the zones of maturation and of hypertrophy was poorly defined (Fig. 1). In the metaphysis these were cartilaginous bars surrounded by osteoid, and numerous osteoclasts were found around the metaphyseal trabeculae (Table 2). The spaces between metaphyseal trabeculae were filled with a large amount of connective and preosteogenic tissue. In the diaphysis these were many hypertrophied osteocytes in wide lacunae.

Treatment with 1-hydroxyvitamin D_3 together with 24,25-dihydroxyvitamin D_3 completely cured the rickets, and the bones were indistinguishable from the control (Fig. 2, Table 2). Treatment with 24,25-dihydroxyvitamin D_3 also prevented rachitic bone lesions. Epiphyses were of the same length and morphology as in vitamin-D_3-treated chicks (Table 2, Fig. 3). In the metaphysis, however, there were fewer trabeculae compared to controls, but the diaphysis appeared normal in width. Abundant haemopoietic tissue was found between the metaphyseal trabeculae, and the number of metaphyseal osteoclasts was slightly greater (Table 2). Osteocytic hypertrophy was also seen in some diaphyseal trabeculae (Fig. 4). These findings, not characteristic of rickets, seemed to be a reflection of the lower plasma calcium levels.

Our study shows that in young growing chicks a combined treatment of the two renal dihydroxylated metabolites of vitamin D can prevent the bone changes seen in rickets, and that the bones formed in such birds are indistinguishable from those formed in birds treated with vitamin D_3. 24,25-Dihydroxyvitamin D_3 alone seems to be sufficient to prevent rachitic bone lesions, at least those affecting cartilage, but probably because of low levels of plasma calcium in our chicks, we saw some changes in the treated bones (fewer metaphyseal trabeculae than normal, together with osteocytic hypertrophy). Based on these findings and the observation that treatment with 1-hydroxyvitamin D_3 as the only source of vitamin D in chicks cannot prevent rachitic changes in bones in spite of normal levels of plasma calcium and inorganic phosphate (unpublished), we propose a function for 1,25-dihydroxyvitamin D and for 24,25-dihydroxyvitamin D_3; we suggest that 1,25-dihydroxyvitamin D_3 increases the calcium and phosphate levels of the extracellular fluid to a supersaturated state in which conditions, in the presence of 24,25-dihydroxyvitamin D_3 which probably acts directly on bone, mineralisation of bone matrix will occur. The possibility that the beneficial effect of 24,25-dihydroxyvitamin D_3 in the presence of 1-hydroxyvitamin D_3, is due to the formation of 1,24,25-trihydroxyvitamin D_3 was ruled out, as it was found that in similar conditions, using tritiated 24,25-hydroxyvitamin D_3, 1,24,25-trihydroxyvitamin D_3 could not be detected in intestine kidney or bone. The exact role that 24,25-dihydroxyvitamin D_3 plays in this process remains to be established.

Fig. 3 Tibial epiphysis from a chick treated with 24,25-dihydroxyvitamin D_3. Note normal arrangement of proliferating, maturing and hypertrophic zones. (Haematoxylin and eosin × 120.)



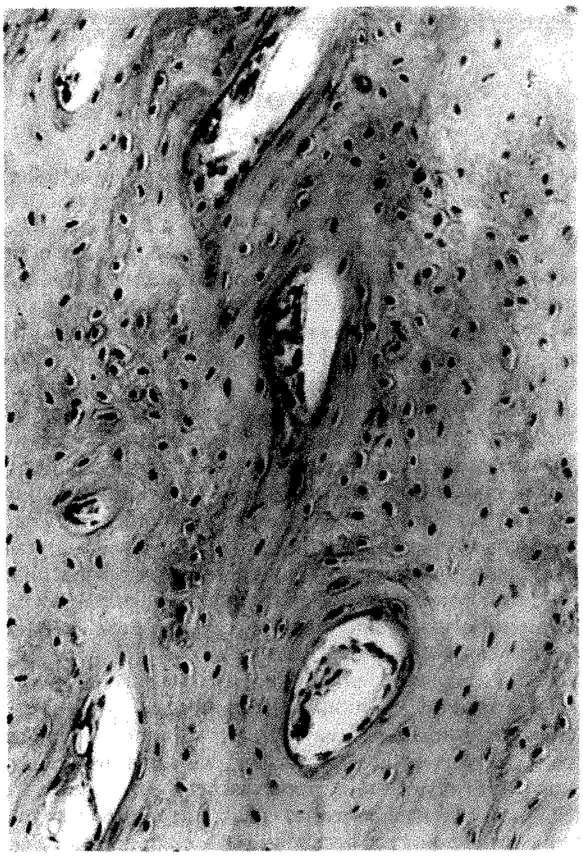


Fig. 4 Tibial diaphyseal trabecula to show slight osteocytic hypertrophy. This chick was treated with 24,25-dihydroxyvitamin D_3. (Haematoxylin and eosin × 250.)

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Ectopic dendritic growth in mature pyramidal neurones in human ganglioside storage disease

PYRAMIDAL CELLS are highly differentiated cortical neurones whose morphophysiological properties derive largely from dendritic systems that provide most of the postsynaptic sites for excitatory and inhibitory synaptic inputs[1]. As pyramidal cell function is closely linked to dendritic geometry[2,3] factors influencing dendritic growth determine to a large extent connectivity patterns in cortical neuronal organisations[4-6]. Although various experimental manipulations hinder attainment of normal dendritic geometry[7] no factors or conditions are known to induce dendritic growth and differentiation in intact, mature cortical neurones. I present here morphological evidence that in human ganglioside storage disease pyramidal neurones, having completed an initial period of normal dendritic growth, undergo a second phase of dendritic growth and differentiation at aberrant sites. I have referred to this in another context as ectopic dendrogenesis[8].

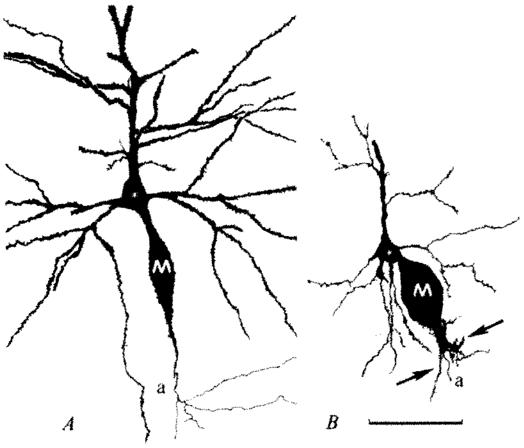

Fig. 1 Changes in abnormal growth characteristics of cortical pyramidal neurones in human ganglioside storage disease, G_{M2}-gangliosidosis (AB variant). Camera lucida drawings of Golgi preparations. *A*, Early stage; onset of seizures and developmental retardation. Neurone as seen in brain biopsy tissue. Reproduced by permission[9]. Dendrites truncated for purposes of illustration. Scale bar 75 μm. *B*, Terminal stage neurobehavioural deterioration, 30 months after observations in (*A*), postmortem tissue. Compare relative size of cell bodies (asterisks) and meganeurites (M) at the two stages. Atrophy of the normal dendritic system and robust growth of aberrant dendrites from the meganeurite (at arrows) occur late in the gangliosidosis. a, Axon. (*A*) is reproduced from ref. 9.

Observations were made initially on cortical pyramidal neurones from a 14-month-old child who presented with seizures and mild neurobehavioural retardation[9]. The diagnosis of G_{M2}-gangliosidosis, AB variant, was established by biochemical analysis of brain biopsy tissue[10]. Golgi studies at the time of the diagnostic biopsy revealed two features of pyramidal neurone morphology. These features are apparent in Fig. 1*A*; Fig. 1*B* shows postmortem findings. The neurone in Fig. 1*A* has well developed and normal appearing dendritic systems as judged by other developmental data on neurones of immature human cerebral cortex[11]. The most significant finding reported originally was the presence of large, fusiform spine-bearing structures, meganeurites (M in Fig. 1*A*) interposed between the cell body and initial portion of the axon. Meganeurites have

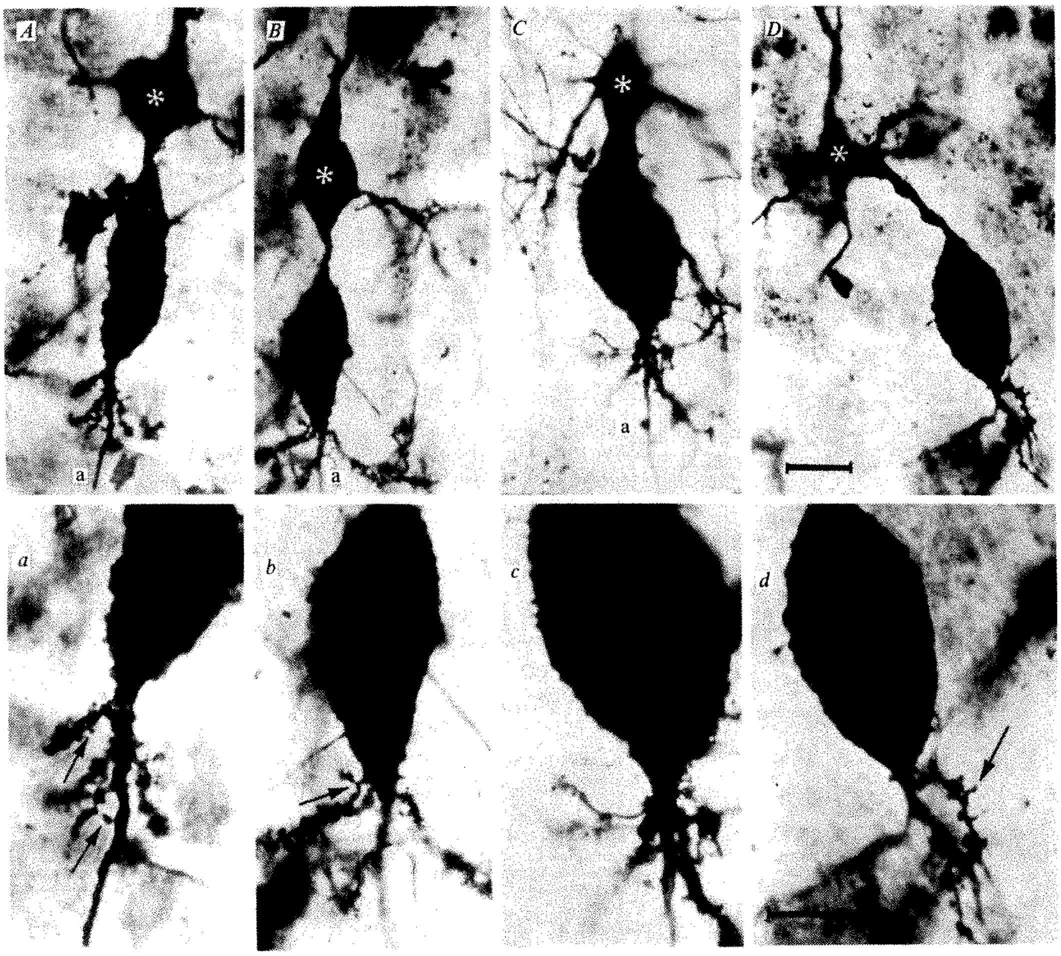

Fig. 2 *A–D*, Examples of medium sized pyramidal neurones at the terminal stage of G_{M2}-gangliosidosis (AB variant) in a 44-month-old child. Golgi preparations of postmortem brain tissue. Cell bodies (asterisks) give rise to variable processes that become massive fusiform or oval structures, meganeurites. Most meganeurites are covered with spines, shown more clearly at higher magnification (*a–d*). Dendrites with dendritic spines (at arrows in *a*, *b* and *d*) arise from the meganeurites. a, Axon. Scale bars, 20 μm.

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since been found in several human ganglioside storage disorders[9] and in feline G_{M1}-gangliosidosis[12].

Between initial diagnosis and death, 30 months later, the child's clinical condition deteriorated in a manner similar to that seen in classical Tay-Sachs disease. Examination of Golgi preparations of postmortem brain tissue (Fig. 1*B*) showed striking changes in pyramidal cells from the first observations 30 months earlier (Fig. 1*A*). Extraordinary growth of meganeurites and atrophy of perisomatic dendritic systems are evident as described in classical Tay-Sachs disease[9]. A significant finding is the outgrowth from the meganeurite of robust dendrites bristling with dendritic spines (Fig. 1*B*, at arrows). Further examples of small and medium pyramidal neurones with prominent dendrites arising from meganeurites are shown in Fig. 2. Several meganeurite-related dendrites in Fig. 2(*a–d*) exhibit a variety of spine shapes characteristic of normal dendritic systems of mature pyramidal neurones[13].

Golgi impregnations of cortical pyramidal neurones of postmortem tissue establish that the aberrant structures designated meganeurites give rise to large spine-covered processes that are identical in every respect to spiny dendrites. Since at an early stage in the course of the ganglioside storage disease studied here meganeurites are prominent but devoid of neurites or dendrites, it follows that growth of meganeurite related dendrites occurred after the period of initial normal dendritic development. The finding that meganeurites serve as 'pseudosomas' for the new dendritic systems confirms previous indications that meganeurites continue to exhibit embryonic growth characteristics that are not expressed by the rest of the ganglioside-laden neurone[9,12,14]. The mechanism by which abnormal ganglioside metabolism signals the genetic machinery of the neurone to synthesise spine-rich dendritic-like membrane for incorporation into meganeurites and related dendrites is unknown. The unprecedented findings here of aberrant dendritic growth induction in otherwise mature human cortical pyramidal neurones suggests that the pursuit of factors associated with abnormal ganglioside metabolism will shed light on the developmental mechanisms of neurone shape regulation and dendritic membrane distribution.

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α- And β-adrenoreceptor blockers have opposite effects on energy metabolism of the central auditory system

THE autoradiographic ^{14}C-deoxyglucose assay has made it possible to measure the local rates of glucose utilisation (LCGU) simultaneously in all the macroscopic components of the central nervous system[1]. Because of the close relationship between local cerebral functional and metabolic activities, the method has proved useful in mapping the distribution of alterations in cerebral functional activity in experimental physiological states[2], and to identify components of cerebral dopaminergic systems in studies with the dopamine agonists, apomorphine[3] and amphetamine[4]. Noradrenergic systems, including both α- and β-adrenergic receptors, have been identified in brain and implicated in various behavioural and affective states in man and animals[5,6]. Drugs with α- or β-adrenergic blocking actions exhibit effects on mood and behaviour[5,6]. Propranolol, a β-adrenergic blocking agent, has, in fact, been used as a therapeutic agent in the control of psychotic symptoms[7,8]. In an attempt to localise the regions of the brain in which functional and/or metabolic activities are altered by adrenergic blockade, we have used the ^{14}C-deoxyglucose method to study the effects of the β-adrenergic blocker, DL-propranolol, and the α-adrenergic blocker, phentolamine. The results demonstrate strikingly opposite effects of these drugs in the auditory system of the rat.

Normal male Sprague-Dawley rats (350–400 g) were catheterised through the femoral artery and vein under halothane anaesthesia. A loose-fitting abdominal-pelvic plaster cast was applied to the animal for restraint, and the animal was allowed to recover from the effects of anaesthesia for at least 2 h. The drugs were then administered by continuous intravenous infusion. Controls were infused with normal saline at 0.04 ml per min; drug-treated animals received equivalent amounts of saline containing either DL-propranolol-HCl or phentolamine mesylate. Propranolol concentration in the saline infusion medium was adjusted to provide a rate of infusion of 0.25, 0.5 or 1.0 mg of drug per kg body weight per min; the concentration of phentolamine was adjusted to 0.25, 0.5 or 0.85 mg per kg per min. The infusions were maintained for 40 min before administration of ^{14}C-deoxyglucose and continued during the subsequent 45 min required for measurement of local cerebral glucose utilisation. The period of measurement of cerebral glucose utilisation was initiated by administration of an intravenous pulse of 125 μCi 2-deoxy-D-[1-^{14}C]glucose (50–55 mCi mmol^{-1}) per kg body weight. Timed arterial blood samples were then drawn at predetermined intervals during the next 45 min (ref. 1). The blood samples were immediately centrifuged, and the plasma was separated and assayed for ^{14}C-deoxyglucose and glucose concentrations as previously described[1]. Approximately 45 min after the pulse the animal was decapitated, and the brain was removed, frozen in Freon XII at −60 to −70 °C for sectioning and autoradiography[1]. Local tissue concentrations of ^{14}C were determined by densitometric analysis of the autoradiograms[1]. Local cerebral glucose utilisation was calculated from the time courses of the plasma ^{14}C-deoxyglucose and glucose concentrations and the local tissue concentrations of ^{14}C by means of the operational equation of the ^{14}C-deoxyglucose technique[1].

Administration of propranolol was associated with changes in several systemic physiological variables. Pulse rate declined; body temperature tended to fall by as much as 2 °C; and arterial P_{CO_2} and P_{O_2} tended to rise. During the administration of the propranolol all the animals showed a gradual decrease in alertness and responsiveness to tactile and auditory stimulation. Propranolol also produced dose-dependent decreases in local cerebral glucose utilisation. With increasing dosage more and more cerebral structures exhibited statistically significant

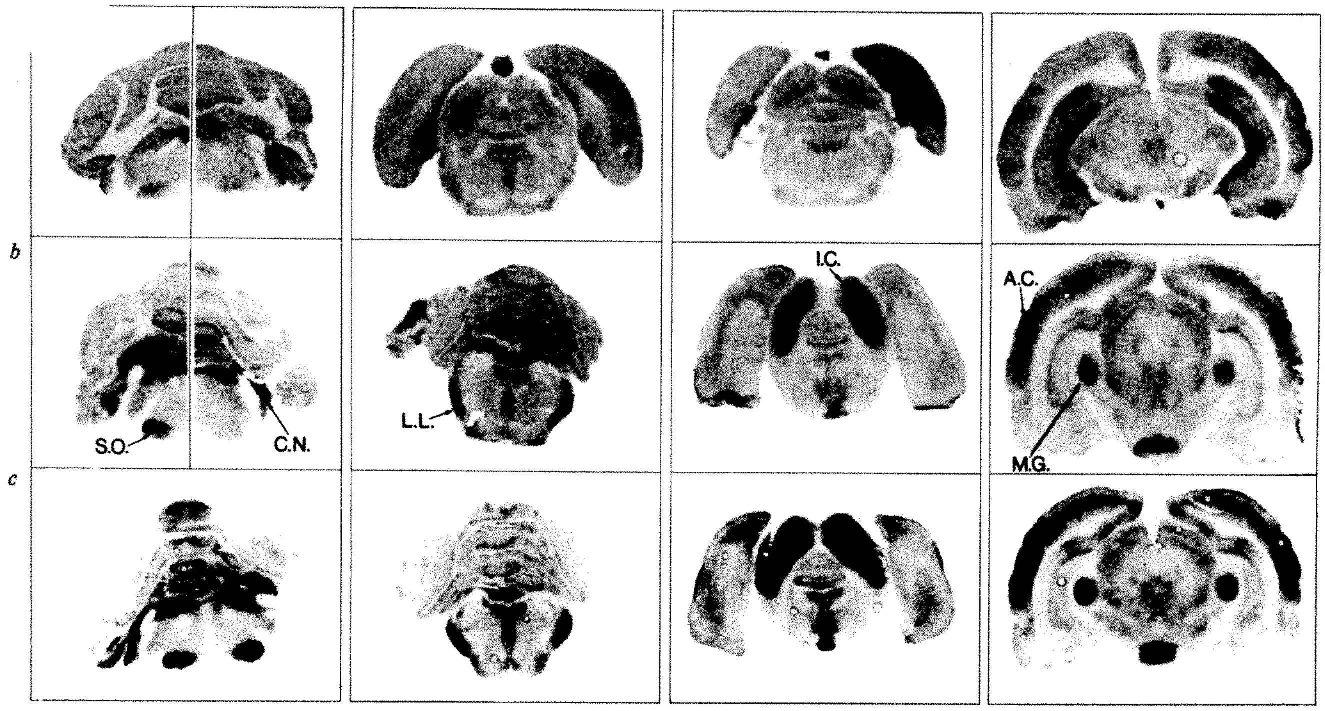

Fig. 1 Autoradiographs of brain sections from three rats illustrating effects of propranolol and phentolamine on glucose utilisation of the auditory system. The sections were at levels that include all components of the auditory system. *a*, Sections from rat given DL-propranolol-HCl 1 mg per kg per min. *b*, Sections from control rat given normal saline. *c*, Sections from rat administered phentolamine mesylate 0.85 mg per kg per min. C.N., cochlear nucleus; S.O., superior olive; L.L., nuclei of lateral lemniscus; I.C., inferior colliculus; M.G., medial geniculate body; A.C., auditory cortex.

Table 1 Effects of DL-propranolol and phentolamine on local glucose utilisation in grey matter of the conscious rat

Cerebral structures	Control (8) (µmol per 100 g per min)	DL-Propranolol* (4)		Phentolamine* (6)	
		(µmol per 100 g per min)	% Effect	(µmol per 100 g per min)	% Effect
Auditory system					
Cochlear nucleus	137±6	47±3†	−66	168±8‡	+23
Superior olivary nucleus	134±7	52±4†	−61	179±7‡	+34
Nucleus of lateral lemniscus	104±7	42±2†	−60	128±7†	+23
Inferior colliculus	181±11	47±5†	−74	228±23	+26
Medial geniculate body	110±4	48±7†	−56	125±7	+14
Auditory cortex	154±5	64±7†	−58	170±13	+10
Miscellaneous grey matter					
Visual cortex	101±3	57±3†	−44	94±6	− 7
Parietal cortex	104±5	56±4†	−46	95±6	− 9
Sensory cortex	108±4	55±3†	−49	98±6	− 9
Olfactory cortex	96±3	45±3†	−53	95±4	− 1
Frontal cortex	105±4	54±5†	−49	101±11	− 4
Thalamus: lateral nucleus	92±4	48±9†	−48	94±8	+ 2
Lateral geniculate body	63±2	36±4†	−43	68±2	+ 8
Lateral hypothalamus	44±1	32±3†	−27	40±1	− 9
Hippocampus	70±2	57±3‡	−19	66±3	− 6
Dentate gyrus	54±2	59±4	+ 9	51±3	− 6
Amygdala	40±2	29±3‡	−28	37±2	− 8
Caudate putamen	99±3	56±10†	−43	103±8	+ 4
Nucleus accumbens	67±3	42±8†	−37	63±3	− 6
Globus pallidus	46±2	30±6‡	−35	42±3	− 9
Substantia nigra	52±3	50±7	− 4	41±1†	−21
Vestibular nucleus	110±3	57±6‡	−48	106±7	− 4
Superior colliculus	77±3	40±3‡	−48	74±5	− 4
Pontine gray	51±3	28±4‡	−45	46±3	−10
Cerebellar cortex	46±2	29±4‡	−37	41±3	−11
Cerebellar nucleus	89±3	48±4‡	−46	88±6	− 1

The values are the means ± s.e.m. obtained in the number of animals indicated in parentheses.
* DL-Propranolol-HCl: 1 mg per kg per min; phentolamine mesylate: 0.85 mg per kg per min.
† $P < 0.01$ by method of Bonferroni *t* statistics[23].
‡ $P < 0.05$.

reductions in glucose utilisation and to a greater degree. At the highest dosage (1 mg per kg per min) glucose utilisation was significantly depressed in all but 2 of the 26 structures examined (Table 1). Although the magnitude of the effects was substantial in many structures, the greatest depressions were in all the components of the auditory system, (cochlear nucleus, superior olive, nuclei of lateral lemniscus, inferior colliculus, medial geniculate body, and auditory cortex, Table 1). Normally, these structures have relatively high rates of glucose utilisation in the brain and, therefore, clearly stand out in the autoradiographs as areas of high optical density (Fig. 1*b*). Propranolol reduced the rates of metabolism of the auditory structures more than those of other structures which caused their representation in the autoradiographs to fade into the background of the other structures (Fig. 1*a*).

The α-adrenergic blocker phentolamine also produced systemic physiological effects. Body temperature tended to fall by as much as 3 °C, and blood pressure and pulse rate fell significantly with all doses used. Most of the animals exhibited progressive decreases in alertness and remained quiet with the eyes closed, but they responded to tactile and auditory stimuli. The penetrability of phentolamine through the blood–brain barrier has been reported to be limited[9], but, nevertheless, the drug at the concentrations used did exert effects on the glucose utilisation of several structures of the brain. Metabolic rate tended to be reduced in most of the structures examined, but these effects were of lesser magnitude than those seen with propranolol. The most striking effects, however, were also in the components of the auditory system, but in contrast to the effects of propranolol, glucose utilisation in these structures was stimulated by phentolamine (Table 1). The effects on the auditory system were sufficiently pronounced that they could readily be visualised in the autoradiograms by the markedly increased densities in the regions corresponding to these structures (Fig. 1*c*).

The results of these studies demonstrate that the α- and β-adrenergic blocking agents, phentolamine and propranolol, respectively, have striking and opposite effects on glucose utilisation in the auditory system. These effects on energy metabolism seem to reflect alterations in functional activity. Furlow *et al.*[10] found that doses of propranolol and phentolamine like those used in the present studies produce corresponding effects on auditory evoked responses in the rat; propranolol depresses and phentolamine enhances the amplitude of all components of the evoked auditory responses.

The stimulation of glucose utilisation by phentolamine seems to be dependent on the presence of auditory input. In rats deafened bilaterally by rupture of the tympanic membranes and occlusion of the external auditory canals with wax, phentolamine failed to increase glucose utilisation in the auditory structures significantly above the low levels seen in deafened animals. These results indicate that the effect of phentolamine is manifested only during auditory input and suggest that its effects in the auditory system may be the result of blockade of an α-adrenergic negative feedback pathway in the central auditory system. α-Adrenergic mechanisms have previously been implicated in the auditory system, and noradrenaline-containing nerve terminals have been localised in most of the structural components of the auditory system[11–16].

DL-Propranolol is not only a β-adrenergic blocking agent, but it also has membrane-stabilising properties. Experiments with sotalol, a more specific β-adrenergic blocking agent lacking membrane-stabilising properties[17], indicate that it does not affect LCGU of the auditory system like DL-propranolol, but it also failed to produce the same behavioural effects, possibly because of its limited ability to cross the blood–brain barrier[17,18]. L-Alprenolol, another specific β-adrenergic blocker, has effects similar to those seen with propranolol, but this drug also has the membrane-stabilising property. Preliminary studies with the separated isomers of propranolol indicate that they have almost equal effects on the glucose utilisation of the auditory system, despite the fact that L-propranolol is twice as potent a β-adrenergic blocking agent as the D-isomer in the central nervous system[19]. Both, however, are approximately equally potent in membrane stabilisation[20], and these results suggest that the membrane-stabilising properties might be responsible for the effects in the auditory system.

Propranolol has recently been proposed as a therapeutic agent for treatment of psychoses[7,8] and is particularly effective in suppressing the auditory hallucinations in schizophrenia[7,21]. Atsmon *et al.*[22] have reported a case study in which the administration of an α-adrenergic blocking agent reversed the beneficial effects of propranolol in a psychotic patient. The effects of propranolol on the auditory system observed in the present studies might be related to its effects in the control of auditory hallucinations.

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High-affinity enkephalin-degrading peptidase in brain is increased after morphine

CONSIDERABLE evidence now exists to suggest that the endogenous opioid pentapeptides Met-enkephalin (Tyr-Gly-Gly-Phe-Met) and Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu) function as neurotransmitters in the central nervous system. A correlate of this hypothesis is that a specific inactivation mechanism must operate in the vicinity of opiate receptors to turn off rapidly the enkephalin signal. Indeed recent studies have shown that the pentapeptides are subject to extremely rapid inactivation in various tissues, occurring primarily by cleavage of the Tyr–Gly amide bond[1–5]. This feature accounts for the short-lasting biological activity of these peptides, contrasting with the potent activity of synthetic analogues such as (D-Ala^2)-Met-enkephalinamide in which this bond is protected[6]. However, many tissues including brain contain a spectrum of peptidases with low specificities and affinities[7] and no evidence has been yet provided for the involvement of a specific

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enzyme in the regulation of enkephalinergic transmission. We now report the presence of a high-affinity peptidase in a particulate fraction of mouse striatum splitting the Leu-enkephalin molecule with release of a tripeptide fragment (Tyr-Gly-Gly) and exhibiting definite substrate specificity. The marked and selective increase in the activity of this peptidase in the striatum of mice chronically treated with morphine suggests that it might be associated with enkephalinergic transmission.

Enkephalin-hydrolysing activity exhibits a considerable heterogeneity in rat brain subfractions[8]. Starting from the hypothesis that a specific peptidase involved in the control of enkephalin concentration in the synaptic cleft should be membrane-bound and should exhibit a high affinity for the pentapeptides, we have compared the saturation kinetics of Leu-enkephalin-hydrolysing enzymes in a soluble and a particulate fraction from mouse striatum, a region containing a high concentration of enkephalins and opiate receptors. As shown in Fig. 1 the shape of the curves representing the fraction of ^{3}H-Leu-enkephalin hydrolysed as a function of total substrate concentration is monophasic in the soluble fraction but clearly biphasic in the particulate fraction. In the particulate, at least two classes of enkephalin-hydrolysing activities are present; one seems to correspond to that found in the soluble fraction ($IC_{50} \sim$ 30 µM in both cases), whereas the other, representing 20–25% of the total hydrolysing activity at low enkephalin concentrations, is restricted to the particulate fraction. This component, represented in Lineweaver–Burk plots (Fig. 1*b*) exhibits a high affinity ($K_m = 90$ nM) as compared to most peptidases for which the K_m is in the range 10–100 µM (ref. 9).

Table 1 Inhibitory potencies of various compounds on the hydrolysis of ^{3}H-Leu-enkephalin by particulate and soluble fractions of mouse striatum

	IC_{50} (nM) for ^{3}H-Leu-enkephalin hydrolysis by Particulate fraction		Soluble fraction
	High affinity peptidase	Low affinity peptidase	
Leu-enkephalin	70	20,000	35,000
Met-enkephalin	350	17,000	39,400
(D-Phe^4)Met-enkephalin	>1,000	25,000	66,000
(L-Ala^2)Met-enkephalin	10	1,000	2,700
(D-Ala^2)Met-enkephalin	>10,000	500,000	520,000
(L-Ala^3)Met-enkephalin	97	9,700	16,500
(D-Ala^3)Met-enkephalin	>1,000	29,000	24,200
Tyr	>100,000	>100,000	>100,000
Tyr-Gly	>100,000	>100,000	>100,000
Tyr-Gly-Gly	>100,000	>100,000	>100,000
Tyr-Gly-Gly-Phe	>100,000	>100,000	>100,000
Gly-Gly-Phe-Met	1,500	>100,000	>100,000
Gly-Gly-Phe-Leu	2,100	>100,000	>100,000
Phenylmethylsulphonylfluoride	>100,000	>100,000	>100,000
Bacitracin	20,000	20,000	20,000
p-Chloromercuriphenylsulphonic acid	>100,000	1,000*	300†
Substance P	>1,000	2,000	1,800
Bradykinin	>1,000	9,000	7,500
Glutathione	>100,000	>100,000	>100,000
Carnosine	>100,000	>100,000	>100,000
LHRH	>100,000	>100,000	>100,000
TRH	>100,000	>100,000	>100,000
α-Endorphin	>5,000	54,000	56,000
β-Endorphin	>5,000	62,000	32,000
Morphine	>100,000	>100,000	>100,000
Naloxone	>100,000	>100,000	>100,000

Particulate and soluble fractions from mouse striata were prepared as described in Fig. 1. Incubations (20°C, 15 min) in the presence of 20 nM ^{3}H-Leu-enkephalin and increasing concentrations (6–8) of the various compounds. ^{3}H-Leu-enkephalin hydrolysis evaluated by column chromatography on Porapak Q beads (see Fig. 1). IC_{50} values were determined by iterative analysis of the concentration-inhibition curves, analogous to that reported in Fig. 1, by a computer program based on the least squares (variance-covariance matrix)[13]. This analysis provided an accurate determination of IC_{50} for the inhibition of the two peptidasic activities in the particulate fraction in the case of a clear biphasic curve, that is, when the IC_{50} differed by more than 50-fold. This was not the case for several compounds: (D-Phe^4)Met-enkephalin, (D-Ala^3) Met-enkephalin, bradykinin and substance P, for which a clear plateau was not present and therefore the IC_{50} on the high-affinity peptidase could not be estimated with accuracy.

* Maximal inhibition ~70%.

† Maximal inhibition ~90%.

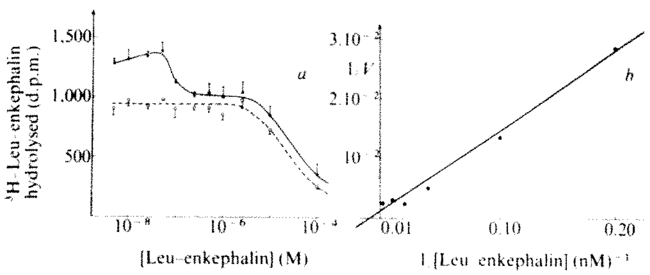

Fig. 1 ^{3}H-Leu-enkephalin hydrolysed by particulate and soluble fractions from mouse striatum incubated in the presence of increasing concentrations of non-radioactive Leu-enkephalin. Striata of three male Swiss mice (18–20 g) were homogenised into 10 ml of 0.05 M Tris-HCl buffer *p*H 7.4 (3 × 5 s at 1,500 r.p.m. in a glass–Teflon Potter homogeniser, clearance 0.1–0.15 mm). A pellet was obtained after two successive centrifugations at 4°C (1,000 *g* × 1 min and 200,000*g* × 1 min) and the supernatant of the second centrifugation was kept as 'soluble fraction'. The pellet was resuspended using a Dounce homogeniser (clearance 20–55 µm) into 10 ml of cold buffer and centrifuged (200,000*g* × 1 min). The resulting pellet, after a wash without resuspension with 15 ml of buffer (which was then discarded), was resuspended into 5 ml of buffer: this suspension was used as 'particulate fraction'. After a 15-min preincubation at 20°C, incubations (15 min, 20°C) of the particulate or soluble fractions were started by addition of 100 µl of buffer containing ^{3}H-Leu-enkephalin (1 nM final concentration; the Radiochemical Centre, 41Ci mmol^{-1}, previously purified by chromatography on Porapak beads microcolumns) and stopped by the addition of 50 µl 0.1 M HCl followed by heat deproteinisation (95°C, 15 min). ^{3}H-metabolites were separated from intact ^{3}H-Leu-enkephalin by chromatography[5] on 80 mg Porapak Q (100-120 mesh, Waters Assoc.) contained in Pasteur pipettes. The metabolites were eluted from column with 2 × 1 ml distilled water directly collected into scintillation vials; Leu-enkephalin was retained on the column. *a*, Effects of increasing concentrations of non-radioactive Leu-enkephalin on the formation of hydrolysis ^{3}H-products by the particulate fraction (●) or the soluble fraction (○). *b*, Lineweaver–Burk plot of the high-affinity Leu-enkephalin hydrolysing activity (corresponding to substrate concentration below 5 µM, that is, up to the plateau) of the particulate fraction (same data as in *a*). The rate of hydrolysis (fmol per mg protein per min) was evaluated, taking into account the specific activities of the substrate. $K_m = 90$ nM, $V_{max} = 850$ fmol per mg protein per min. Means ±s.e.m. of three determinations.

In six independent experiments the presence of this high-affinity component was confirmed and the analysis of the data by a computer program (see Table 1 legend) led to mean K_m values (±s.e.m.) of 41 ± 10 nM and of 55,000 ± 17,000 nM for the high- and low-affinity peptidase activities, respectively. Both peptidase activities in the particulate fraction vary linearly as a function of protein concentration or time.

The high-affinity of a class of peptidase activity found in the particulate fraction suggested that it might exhibit substrate specificity. This was indirectly investigated by determining the inhibitory potency of a variety of compounds towards the different classes of peptidase activities (Table 1). The specificity of the high-affinity peptidase activity is demonstrated by the enormous range in inhibitory potency of the various compounds tested (IC_{50} between 10 and >100,000 nM). Although the low-affinity peptidase activities in both particulate and soluble fractions also exhibit some specificity, the range in IC_{50} variation is much less pronounced. The main conclusions which can be drawn from the data of Table 1 can be summarised as follows:

(1) Stereochemical requirements are more stringent in the case of the high-affinity peptidase as evidenced for the pair of (Ala^3)-Met-enkephalin stereoisomers.

(2) The integrity of the enkephalin molecule is important for the inhibitory potency as shown by the dramatic loss of activity towards all classes of peptidases in the fragments Tyr, Tyr-Gly, Tyr-Gly-Gly and Tyr-Gly-Gly-Phe.

(3) The C-terminal moiety of the enkephalin molecule seems to be more important for inhibition of the high-affinity peptidase

Table 2 Identification by thin-layer chromatography of the radioactive compounds present after incubation of ^{3}H-Leu-enkephalin with a particulate fraction of mouse striatum

Solvent system	Incubation conditions	^{3}H-compounds (d.p.m.) Before column chromatography Tyr	Tyr-Gly-Gly	Leu-enkephalin	After column chromatography Tyr	Tyr-Gly-Gly
(1)	Control$^{(a)}$	1,526 ± 101	628 ± 91	14,840 ± 122		
	+Leu-enkephalin$^{(a)}$ (5 μM)	1,217 ± 171	291 ± 42**	15,524 ± 188*		
	Control	418 ± 90	465 ± 18	15,880 ± 693	500 ± 55	705 ± 58
	+Gly-Gly-Phe-Met (10 μM)	303 ± 43	118 ± 30***	16,503 ± 518	575 ± 65	265 ± 83*
(2)	Control	838 ± 110	530 ± 33	16,305 ± 478	998 ± 45	955 ± 65
	+Gly-Gly-Phe-Met (10 μM)	743 ± 15	100 ± 10***	17,545 ± 555	895 ± 15	293 ± 18***
(3)	Control				570 ± 50	660 ± 53
	+Gly-Gly-Phe-Met (10 μM)				555 ± 88	215 ± 18***

Incubation of samples (30 μg proteins per 200 μl) of the particulate fraction (15 min, 20°C) in the presence of 250 nM of purified ^{3}H-Leu-enkephalin and 1 μM *p*-chloromercuriphenylsulphonic acid (except in conditions (a)) and in the presence or absence of either Leu-enkephalin or Gly-Gly-Phe-Met. A 10 μl aliquot of the deproteinised supernatant or of the Porapak column eluate was applied on a TLC plate (plastic sheets Silicagel 60, Merck, thickness 0.2 mm). Solvent systems and R_f values of the reference compounds were: (1) $CHCl_3:CH_3OH:AcOH:H_2O$ (45:30:6:9); Tyr = 0.61, Tyr-Gly-Gly = 0.47, Leu-enkephalin = 0.88 (2) $BuOH:AcOH:H_2O$ (4:1:1); Tyr = 0.47, Tyr-Gly-Gly = 0.31, Leu-enkephalin = 0.58 (3) isopropanol : AcOEt : 5% AcOH (2:2:1); Tyr = 0.70, Tyr-Gly-Gly = 0.41, Leu-enkephalin = 0.80. Markers visualised with a ninhydrin spray and radioactive peaks at their R_f isolated by cutting the plates and counted by liquid scintillation (40% efficiency). Means ±s.e.m. of 3–6 determinations * $P < 0.025$. ** $P < 0.01$. *** $P < 0.001$. No other radioactive peak could be detected when the entire plate was counted by fractions of 0.5 cm. In particular no significant radioactivity was detected at the R_fs of Tyr-Gly-Gly-Phe (0.80 in system (1) and 0.45 in system (2)).

Table 3 Effect of chronic morphine treatment on ^{3}H-enkephalin-hydrolysing activity of particulate and soluble fractions of mouse striatum

	^{3}H-enkephalin-hydrolysing activity (d.p.m. per mg protein per min) Particulate fraction High affinity peptidase	Low affinity peptidase	Total peptidase	Soluble fraction
Controls	7,858 ± 977	33,344 ± 2,309	41,203 ± 2,620	120,990 ± 20,468
Morphine	12,521 ± 932** (+59%)	33,255 ± 1,998^{NS}	45,776 ± 2,042* (+12%)	125,297 ± 9,590^{NS}

Groups of mice were implanted under light ether anaesthesia with a morphine pellet (75 mg of base, s.c.) which was removed 3 days later, 16 h before death. Controls were sham-operated in a parallel manner. ^{3}H-enkephalin-hydrolysing activity measured after incubations of fractions prepared as described in Fig. 1 (15 min at 20°C) with 20 nM ^{3}H-Leu-enkephalin. The low-affinity hydrolysing activity of the particulate fraction represents the activity measured in the presence of 5 μM Leu-enkephalin whereas the high-affinity activity was estimated as the difference between total (in the absence of 5 μM Leu-enkephalin) and low-affinity activities. Degraded ^{3}H-Leu-enkephalin was measured by Porapak column chromatography. Means ±s.e.m. of 12–16 experiments on pooled striatal fractions from two mice. * $P < 0.05$. ** $P < 0.0005$. NS, not significant.

than the N-terminal moiety as shown by the much greater potency of the tetrapeptides Gly-Gly-Phe-Met or Gly-Gly-Phe-Leu when compared to the tetrapeptide Tyr-Gly-Gly-Phe; this conclusion does not hold true for the two classes of low-affinity peptidases.

(4) Among classical peptidase inhibitors, only bacitracin has significant potency towards the high-affinity peptidase, whereas *p*-chloromercuriphenylsulphonic acid, a potent inhibitor of the low-affinity peptidases, is practically inactive on the high-affinity enzyme; this indicates that the latter does not belong to the group of thiol-peptidases. The heterogeneity of enkephalin-hydrolysing activity as shown by response to thiol-blocking agents has been already suggested[8].

(5) Structural requirements for recognition of the opiate receptors and the high-affinity peptidase are unrelated as demonstrated by the lack of potency of α- and β-endorphin as well as opiates.

From these data it seems that three compounds are useful to differentiate the high- and low-affinity peptidase activities in the particulate fraction: Leu-enkephalin, Gly-Gly-Phe-Met and *p*-chloromercuriphenylsulphonic acid. Therefore, these compounds were used as tools in the identification of the ^{3}H-fragments released under the action of each class of peptidases in the particulate fraction.

Thin-layer chromatography of extracts (obtained after incubation of ^{3}H-Leu-enkephalin with the particulate fraction) yields three radioactive peaks with R_f values closely corresponding to ^{3}H-Leu-enkephalin, ^{3}H-Tyr and ^{3}H-Tyr-Gly-Gly (Table 2). In contrast, only ^{3}H-Leu-enkephalin and ^{3}H-Tyr were found after incubation of the soluble fraction. Whereas the presence of ^{3}H-Tyr is in agreement with a number of reports[1–5], ^{3}H-Tyr-Gly-Gly was an unexpected component. The presence of ^{3}H-Tyr-Gly-Gly was confirmed first by unidimensional TLC in three different solvent systems (Table 2), second by bi-dimensional co-chromatography in solvent systems (2) and (3), and third by high-performance liquid co-chromatography on a 205 U Waters apparatus (reversed phase on standard micro-bondapak C 18; solvent CH_3CN : 10 mM NH_4Ac buffer, 3.125 : 96.875; 1 ml min^{-1}; retention times Tyr = 290 s, Tyr-Gly-Gly = 350 s, Leu-enkephalin > 1 h).

The fact that Tyr-Gly-Gly was not previously identified by others as a breakdown product of enkephalin can easily be explained by its low level relative to total metabolites (~25% in our washed particulate fraction but only 2–3% in the whole homogenate as used by most workers). Nevertheless, the occurrence of a minor unidentified peak with R_f corresponding to Tyr-Gly-Gly has been reported in brain extracts[2], and formation of this tripeptide by cultured human endothelial cells has been reported[10].

That ^{3}H-Tyr-Gly-Gly is specifically produced by the high-affinity peptidase is clearly demonstrated by first the marked decrease observed after incubations in the presence of 5 μM Leu-enkephalin or 10 μM Gly-Gly-Phe-Met contrasting with the unchanged ^{3}H-Tyr peak in the same conditions; and second the lack of effect of *p*-chloromercuriphenylsulphonic acid on ^{3}H-Tyr-Gly-Gly formation, contrasting with the strong reduction in the ^{3}H-Tyr peak (solvent system (1)). These results indicate that the high-affinity peptidase is probably a C-terminal dipeptidase splitting the Gly–Phe bond of enkephalin, a conclusion consistent with the high inhibitory potency of the C-terminal tetrapeptide as compared to the N-terminal tetrapeptide (Table 1). As we have not yet characterised the formation of Phe-Leu or Phe-Met (because they are not labelled) we cannot exclude the possibility that ^{3}H-Tyr-Gly-Gly formation results from the sequential action of carboxypeptidases. However, this is unlikely because ^{3}H-Tyr-Gly-Gly-Phe formation was never observed in our experiments.

In mice chronically treated with morphine we observe a dramatic increase in the activity of the high-affinity peptidase in the particulate fraction (Table 3). A similar effect is also observed in rats implanted with a morphine pellet (data not shown). This effect seems to be selective because the low-affinity peptidase activity in either the particulate or the soluble fractions is unchanged. The increase in activity reflects an elevated V_{max} without significant change in K_m value (data not shown), suggesting that it corresponds to an increased number of enzyme molecules. The effect of morphine might result from a feedback mechanism operating at the target cells bearing the opiate receptors: conceivably these cells, to compensate the overstimulation of opiate receptors, could develop a state of hyposensitivity to released enkephalins, through an increased rate of inactivation of these peptides. In this respect, changes in acetylcholinesterase activity in brain have been already reported to mediate the modified responsiveness of target cells to the transmitter, developing in response to sustained changes in cholinergic transmission[11]. Although not direct proof, the effect of morphine strongly suggests that the high-affinity peptidase is involved in the control of enkephalinergic transmission. This hypothesis is consistent with the heterogeneous distribution of this 'enkephalinase' in mouse brain regions (striatum > hypothalamus > cortex > brainstem ⩾ hippocampus > cerebellum, with a five-fold variation between striatum and cerebellum) which is highly correlated ($r = 0.94$) with that of opiate receptors (manuscript in preparation). If true, this would imply that a new class of therapeutic agents (enkephalinase inhibitors) exhibiting opiate-like activity could be developed. In addition the increased peptidase activity after chronic morphine treatment could be involved, with other mechanisms[12], in the development of dependence on opiates.

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The ACTH 'family tree' of the rhesus monkey changes with development

THE pituitary hormone adrenocorticotrophin (ACTH) belongs to a 'family' of peptides derived from a common 'stem hormone' (Fig. 1). Depending on the way in which the stem hormone is cleaved, the various peptides are expressed and it is possible that qualitative changes in the expression of the peptide 'family tree' may be responsible for the alteration in fetal adrenal function which precedes birth[1]. We now report evidence for such changes in the rhesus monkey, which has a fetal adrenal comparable with that of man.

After delivery the fetal zone of the human adrenal cortex involutes, while the definitive zone or adult cortex, consisting of a thin layer of cells covering the large fetal zone, hypertrophies. This change may be triggered by a switch in the production of trophic hormones in the fetus, from peptides resembling α-melanocyte stimulating hormone (α-MSH) and corticotrophin-like intermediate lobe peptide (CLIP) to ACTH[2,3]. The idea is supported by a report[4] that α-MSH stimulated the fetal adrenal gland of the rabbit when synacthen (α-1–24 ACTH) was relatively ineffective, whereas synacthen was potent in the newborn and α-MSH was ineffective. Others have shown that although ACTH stimulated the definitive zone *in vitro* its effect on the fetal zone was inconsistent[5]. The metamorphosis of the adrenal at birth is but one aspect of the change in adrenal function. In the sheep, for example, in which there is no specific fetal zone, there are nevertheless important changes in steroid output, notably a prepartum rise of cortisol which prepares the fetus for delivery and initiates parturition[6]. As with the human, there are differences in the composition of pituitary hormones between fetus and adult[1]. Unlike the human, however, it is the large molecular weight forms of ACTH which predominate in the fetal sheep. There are three such forms, two of which contain sequences common to β-lipotrophic hormone (β-LPH), γ-LPH,

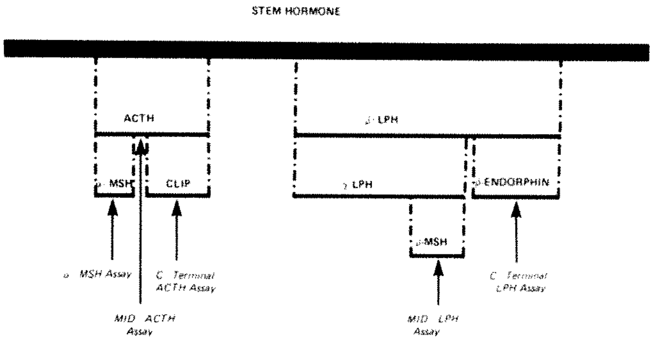

Fig. 1 The stem hormone family tree gives rise to two major branches—ACTH and β-LPH. ACTH is a 39-amino acid peptide whose first 13 amino acids are identical in sequence with α-MSH and whose 18–39 sequence is identical to CLIP. β-LPH is a 91-amino acid peptide whose first 58 amino acids are identical in sequence with γ-LPH and whose 61–91 sequence is identical to β-endorphin. The sequence 41–58 is identical to the 18-amino acid peptide β-MSH. The five radioimmunoassays are directed against different antigenic sites of this family tree.

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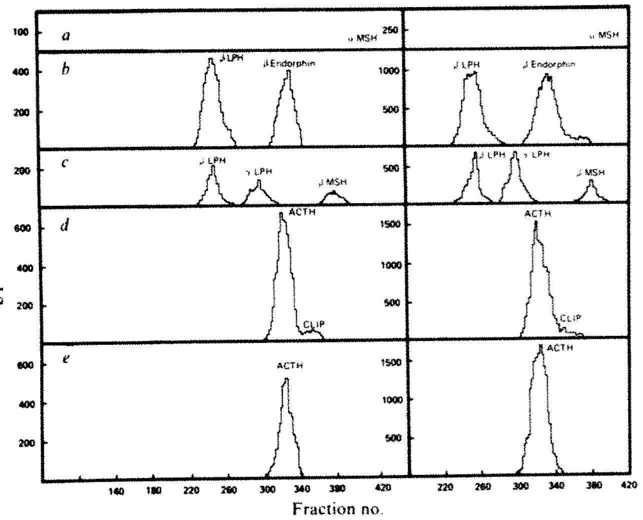
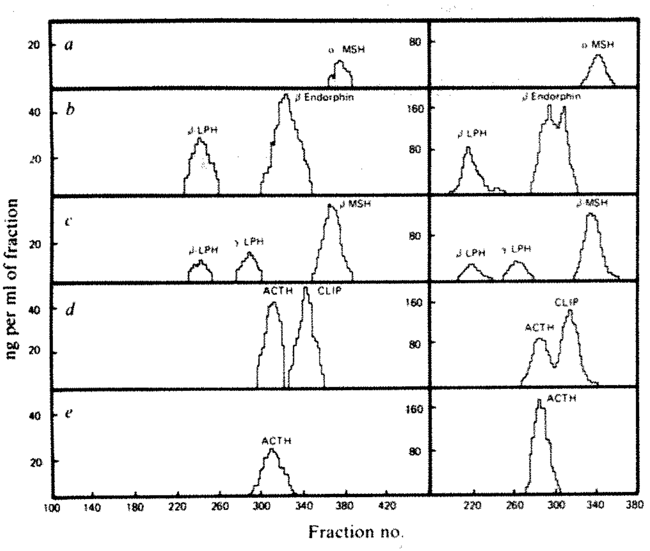


Fig. 2 Pituitary extracts from two adult monkeys (left, infant 77 days postpartum; right, mature adult) individually fractionated on Sephadex G100 superfine: alternate fractions were assayed in five systems of radioimmunoassay. *a*, α-MSH activity; *b*, C-terminal LPH; *c*, mid-LPH; *d*, C-terminal ACTH; *e*, mid-ACTH.

β-MSH, β-endorphin and possibly other biologically active peptides as well as ACTH, α-MSH and CLIP. These precursors are the 'stem hormones'.

We have used the pituitary of the rhesus monkey for further investigations because, in common with man, its adrenal gland has a fetal zone which involutes at birth. Dated pregnant monkeys were anaesthetised with 20 mg phencyclidine hydrochloride (Sennylan, Bioceutics). Fetuses were delivered by hysterotomy, their pituitaries freshly dissected, snap frozen in liquid nitrogen and stored at −70 °C. Pituitaries from newborn, infant, and mature adult monkeys were freshly dissected and stored at −70 °C. Pituitaries were extracted in 0.02M HCl with 10^{-3} M diisopropylphosphofluoridate, loaded on to a column (1.6 × 100 cm) of Sephadex G100 superfine and eluted in 1M acetic acid at 1.6 ml h^{-1}. Fractions were collected every 15 min and alternate fractions assayed in five systems of radioimmunoassay (Fig. 1) directed against different branches of the stem hormone family tree. The α-MSH assay measured only α-MSH and was probably directed against the acetylated or amidated end groups of the molecule. The mid-ACTH assay measured ACTH and did not cross-react with α-MSH, CLIP or the LPH family. The C-terminal ACTH assay cross-reacted with CLIP as well as ACTH. The mid-LPH assay cross-reacted with β-MSH, γ-LPH and β-LPH. The C-terminal LPH assay cross-reacted with β-endorphin and β-LPH.

Fig. 3. Pituitary extracts from two fetal monkeys (left, 145 days gestation; right, 164 days gestation) individually fractionated on G100 superfine: alternate fractions were assayed in five systems of radioimmunoassay. (*a*–*e* as Fig. 2.)



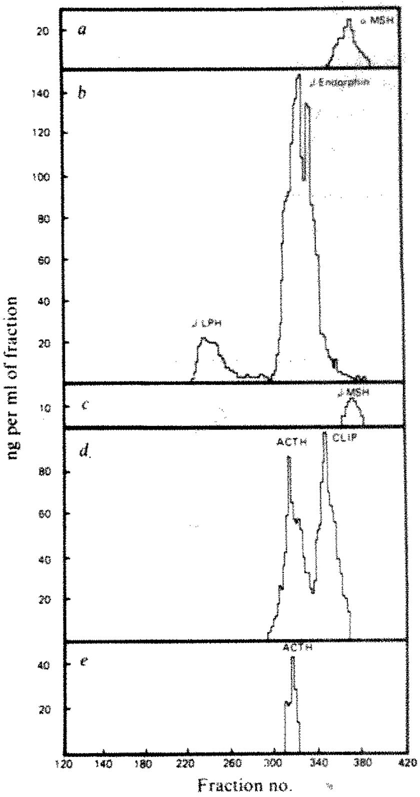


Fig. 4 Pituitary extract from a neonate (12 h postpartum) fractionated on Sephadex G100 superfine: alternate fractions were assayed in five systems of radioimmunoassay. (*a*–*e* as Fig. 2.)

The patterns of peptides seen in the pituitary of an infant and a mature adult were similar (Fig. 2). The major peak in both pituitaries was ACTH with relatively insignificant amounts of α-MSH and CLIP. Further peaks of activity were associated with β-LPH, γ-LPH, β-endorphin and β-MSH. In fetal pituitaries taken at 145 days gestation and 164 days gestation, β-MSH was increased relative to β- and γ-LPH and β-endorphin increased relative to β-LPH (Fig. 3). There was a significant peak of α-MSH and a peak of CLIP which equalled that of ACTH. A pituitary from a neonate killed 12 h after delivery also showed substantial peaks of α-MSH and CLIP compared to ACTH (Fig. 4). However, the major peak was that of β-endorphin which dominated the peaks of β-LPH, γ-LPH and β-MSH.

The fetal and adult pituitaries did not contain substantial amounts of 'big ACTH' or stem hormone. However, small quantities were identified. An extract from a mature adult pituitary showed dominant peaks of ACTH, β-LPH, γ-LPH, β-MSH and β-endorphin, plus three peaks of large molecular weight material 'A', 'B' and 'C' (Fig. 5). Small quantities of peak A were detected by the mid-ACTH assay, peak B cross-reacted in the LPH assays and the ACTH assays, peak C only cross-reacted in the ACTH assays. The position of these three peaks corresponded to material found in abundance in the fetal sheep pituitary, where A and B were characterised as stem hormones and C as big ACTH[1].

These results reveal significant alterations of the family tree at different stages of development of the monkey. As with the human, α-MSH and CLIP predominate in fetal life and all but disappear in the adult. Unlike the human, the pituitary of the rhesus monkey contains β-MSH with relatively greater amounts in the fetus than the adult. The levels of β-endorphin also appear more prominent in fetal life and it may consist of more than one molecular form. The overall picture is that the small molecular weight peptides α-MSH, CLIP, β-MSH and β-endorphin are characteristic of the fetal pituitary of the rhesus monkey, whereas in the adult pituitary the larger peptides ACTH, γ-LPH and β-LPH are the predominant forms.

Previously we have reported the occurrence of small amounts of a common precursor for ACTH and LPH in the human pituitary[7,8] and such a molecule has been identified in a mouse pituitary tumour cell line[9,10]. The stem hormones are found in abundance in the pituitary of the sheep and the fact that they can now be identified, even in small quantities, in the monkey substantiates the hypothesis that there is a family tree of biologically active peptides which derive from a common stem. The change in fetal adrenal function and morphology which precedes parturition must, therefore, be measured against the qualitative changes of the 'trophic family' as a whole.

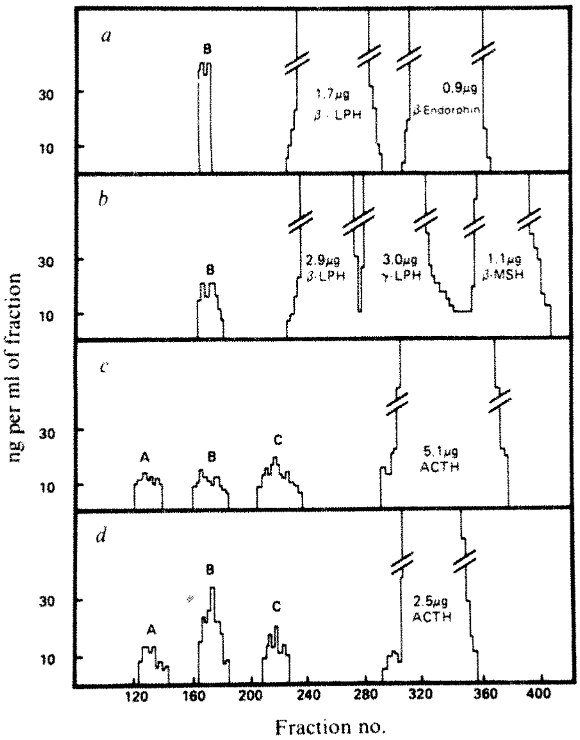


Fig. 5 Pituitary extract from a mature adult monkey fractionated on Sephadex G100 superfine. Alternate fractions were assayed in four systems of radioimmunoassay. In addition to the major peaks of ACTH, β-LPH, γ-LPH, β-endorphin and β-MSH there were three small peaks of large molecular weight material A, B and C. *a*, C-terminal LPH assay; *b*, mid-LPH; *c*, C-terminal ACTH; *d*, mid-ACTH.

There has been considerable speculation about β-endorphin since the dramatic finding that, like enkephalin[11], it can bind to morphine receptors[12–17]. The name suggests that it is endogenous morphine, but there has been little to illuminate its functional role within the mammalian neuroendocrine system. Moreover, the administration of β-endorphin has not always proved successful as an analgesic, except when given directly into the brain ventricles[18,19]. It is an unexpected finding that there is a striking increase of β-endorphin in the pituitary of the newborn monkey (Fig. 4) and this may prove of great physiological importance. Although the moment of birth has been considered a time of intense psychological trauma for the fetus[20,21], it is clearly a moment of extreme physical stress and it is possible that the fetus needs to be rendered insensitive to the assault of parturition. High levels of β-endorphin at delivery may thus serve to protect the baby against a necessary but otherwise intolerable event.

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Differences in fluidity between bilayer halves of tumour cell plasma membranes

SURFACE membranes of both normal and transformed cells appear to have an asymmetric distribution of phospholipids across the bilayer[1–6]. The potential role of this distribution in platelet aggregation and in pathological conditions such as sickle cell anaemia and cell transformation has been investigated[7–9]. However, neither the structural nor functional significance of this asymmetrical distribution is understood at present. As the lipid composition of the two sides of the plasma membrane bilayer is different it seems possible that the 'fluidity' or other physicochemical properties are also different. It has been shown that the neutral zwitterionic lipids (phosphatidylcholine and sphingomyelin) are located primarily in the outer monolayer while the anionic zwitterionic lipids (phosphatidylethanolamine,

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phosphatidylserine and phosphatidylinositol) are located primarily in the inner or cytoplasmic monolayer. In model systems the former lipids are usually more fluid while the latter lipids are more rigid[10]. Thus, a fluidity gradient could exist across the bilayer of biological membranes. Such a gradient and alterations of it may have important consequences in vectorial processes such as small molecule transport, receptor mobility, hormone receptor interactions and the activity of membrane-bound enzymes. However, as mammalian cells can compensate or homeoviscously adapt and because biological membranes are complex mixtures of cholesterol and phospholipids with different fatty acyl chain length or unsaturation, direct experimental measurement is necessary to determine if such vertical asymmetry of 'fluidity' exists across the plasma membrane bilayer of mammalian cells[11–16]. I report here use of a fluorescent probe molecule, β-parinaric acid, used in conjunction with the amino labelling reagent 2,4,6-trinitrobenzenesulphonic acid (TBNS) to investigate this asymmetry. The data obtained are consistent with the conclusion that the inner monolayer of the plasma membrane is less fluid than the outer monolayer.

LM cells (ATCC-CCL 1.2), a strain of transformed murine fibroblasts, were grown in suspension in a chemically defined medium and plasma membranes isolated as described previously[11]. The cells or isolated plasma membranes were labelled with TBNS under penetrating or nonpenetrating conditions as previously stated[5]. The computer-centred spectrofluorimeter of Holland *et al.*[12,13] was used for all fluorescence measurements[14–17].

As β-parinaric acid distributes rapidly across both sides of the plasma membrane, one method to determine the physical properties of the inside monolayer of the plasma membrane would be to quench fluorescence of the probe in one monolayer and measure the fluorescence properties of the probe in the unquenched monolayer. The amino labelling reagent TBNS can be used as such a chemical quencher of β-parinarate fluorescence on one side of the bilayer membrane. Free TBNS has an absorption maximum in aqueous solution at 340 nm. However, after reacting with free amino groups a new absorption band near 420 nm appears[18,19]. This absorption band overlaps with the lower wavelength side of the fluorescence emission spectrum of β-parinaric acid in plasma membranes and in blood lipoproteins and is therefore an ideal quenching agent if the quenching of β-parinarate fluorescence is nontrivial[14–19]. This quenching is nontrivial because the shape of the fluorescence emission curve of β-parinarate in the plasma membrane is unaltered by trinitrophenyl amino groups and the fluorescence of β-parinarate in organic solvents is quenched completely by trinitrophenylglycine, indicating high quenching efficiency of β-parinarate fluorescence by the trinitrophenyl group. If both sides of the plasma membrane were labelled by TBNS then fluorescence of β-parinarate should be quenched almost completely. When both sides of the isolated plasma membrane of LM cells are labelled with TBNS, absorption-corrected fluorescence, relative fluorescence efficiency and corrected fluorescence emission are quenched 91–95% (Fig. 1). Thus, most of the β-parinarate is accessible to quenching by energy transfer. If only the outer monolayer is labelled with TNBS, then these parameters are quenched only 44–46%. These results indicate that the trinitrophenylated amino groups of the outer monolayer cannot quench the fluorescence of β-parinarate in the inner monolayer. The data are also consistent with an approximately equal distribution of β-parinarate across both sides of the bilayer and indicate that the properties of the inner monolayer can be investigated independently of the outer monolayer.

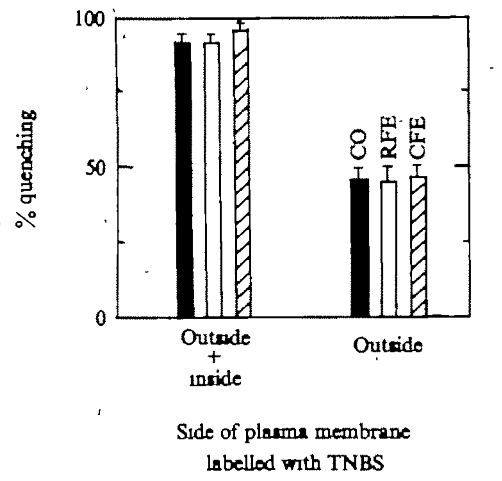

Fig. 1 Fluorescence quenching of β-parinaric acid in isolated LM cell plasma membranes by TNBS. β-parinarate quenching of outside+inside monolayers of the plasma membrane was determined as follows: plasma membranes were isolated as stated previously[11] and reacted under penetrating conditions with TNBS to trinitrophenyl-label all free amino-phospholipids in both monolayers of the plasma membrane as described elsewhere[5]. β-parinarate was incorporated into the plasma membranes at a β-parinarate/lipid molar ratio of 1:100 and absorption corrected fluorescence (CO), relative fluorescence efficiency (RFE), (CFE) and corrected fluorescence emission were determined at 313 nm, 313 nm and 415 nm respectively as described earlier[14–17]. The per cent quenching of β-parinarate was determined by comparison of the values for these fluorescence parameters with values for β-parinarate in equivalent samples that had not been reacted with TNBS. The degree of fluorescence quenching in the outside monolayer was determined by prelabelling the intact LM cells under nonpenetrating conditions with TNBS followed by membrane isolation and incorporation of β-parinarate into the membranes as above[5,14–17]. Values indicate the mean ± s.e.m. ($n = 3$) and differences in each parameter are significantly different at $P < 0.01$ by Student's *t* test. Outside and inside refer to the side of the plasma membrane labelled with TNBS

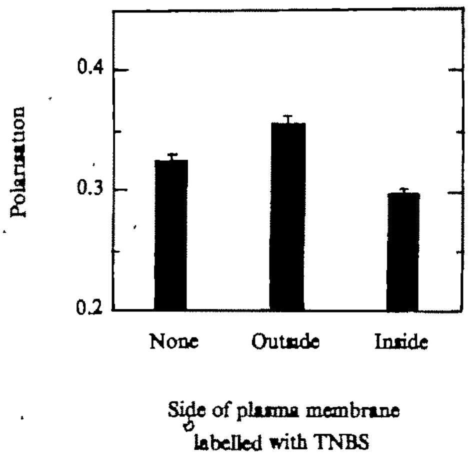

Fig. 2 Asymmetry of fluorescence polarisation between outer and inner monolayers of isolated LM cell plasma membranes. All methods were as described in the legend to Fig. 1 for [outside + inside monolayers] and [inside monolayer]. Polarisation values for the [outer monolayer] were calculated as described in the text. Polarisation was measured as described elsewhere. None, outside and inside refer to the side of the plasma membrane labelled with TNBS. Thus, polarisation values represent the side opposite to the side labelled with TNBS. Each value represents the mean ± s.e.m. ($n = 3$); all values are different at $P < 0.01$ by Student's *t* test.

The asymmetry of 'fluidity' across the plasma membrane bilayer is best demonstrated by the polarisation measurements shown in Fig. 2. Fluorescence polarisation is directly proportional to microviscosity. The polarisation of β-parinarate in plasma membranes that were not reacted with TNBS was 0.325 ± 0.005 while that in plasma membranes labelled on the outside with TNBS was 0.355 ± 0.006. The polarisation of β-parinarate in the outer monolayer can be estimated to be 0.290, using the formula $P_{\text{inside+outside}} = xP_{\text{inside}} + (1-x)P_{\text{outside}}$ (where x

is the mole fraction of β-parinarate), assuming x to be 0.5. This seems to be a reasonable assumption in view of the results shown in Fig. 1. Thus, the minimum difference in polarisation between the inner and outer monolayer would be 0.030 (if almost all of the probes were located in the outer monolayer).

The data presented here indicate that the inner monolayer of plasma membranes from a tumorigenic cell grown in tissue culture may be more rigid (lower fluidity) than the outer monolayer. The function of such a fluidity gradient across the membrane bilayer is not known. However, it is known that both in normal and transformed cells the lipid composition of the membranes can be modified by medium constituents (see ref. 11 for review). Thus, alterations in or regulation of membrane fluidity gradients may be important in membrane pathologies. In support of this, several types of fluidity asymmetries have been suggested by electron spin resonance data. Red blood cell membranes appear to have a more rigid outer monolayer than inner monolayer, a finding opposite to that presented here[20]. In contrast, sarcoplasmic reticulum membranes do not have an asymmetry of physical properties in the hydrocarbon region of the bilayer but an asymmetry similar to that presented here exists for the phospholipid polar head groups across the bilayer[21]. These results may point out basic differences in asymmetry of physical properties of membranes in mammalian cells.

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A direct method for determination of membrane electron density profiles on an absolute scale

THE principal aim in X-ray diffraction studies of biological membranes and lipid bilayers is to determine the electron density profile across the membrane and to interpret this in terms of the distribution of the component molecules. The derivation of such electron density profiles requires a knowled of both the amplitudes of the X-ray reflections and their co responding phase angles. Although the relative amplitudes c be easily determined from a diffraction pattern, the phases of t reflections cannot be directly observed. The determination these phases has proved to be one of the most difficult ar contentious aspects of the membrane diffraction field[1–9]. I addition, because of the difficulty in measuring absolu intensities[10,11], the profiles are, almost invariably, calculated c a relative electron density scale. The lack of an absolute scale h often greatly hindered the interpretation of membrane profile particularly with regard to the distribution of the prote molecules[9,12–16]. We describe here a novel method of direct determining both the phases of the X-ray reflections and a absolute electron density scale, and how the method has bee successfully tested on lecithin/cholesterol bilayers.

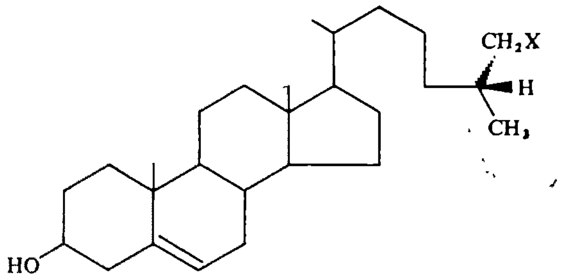

Fig. 1 The cholesterol analogue used to introduce a halogen atom into the bilayer. X is either bromine or iodine. The molecule is identical to cholesterol except for the exchange of a hydrogen atom on C-26 by the halogen. The (25*R*)-26-bromocholesterol, melting point 134–136 °C, NMR δ 3.32 (d, $J = 6$ c.p.s., $-CH_2Br$), was made by treating (25*R*)-26-hydroxycholesterol (prepared from kryptogenin) with *N*-bromosuccinamide and triphenylphosphine in tetrahydrofurane. Similarly, treatment of (25*R*)-26-hydroxycholesterol with *N*-iodosuccinamide and triphenylphosphine in tetrahydrofurane gave the (25*R*)-26-iodocholesterol, melting point 118–120 °C, NMR δ 3.2 (d, $J = 6$ c.p.s., $-CH_2I$).

The basis of the method is the isomorphous exchange cholesterol in the membrane with a cholesterol analogue whi is halogenated at C-26 (see Fig. 1). The introduction of th molecule into the membrane places an electron-dense ato close to the bilayer centre. Figure 2 shows schematically how t introduction of such a heavy atom affects the diffraction patte for the simple case of a unit cell containing a single centr symmetric lipid bilayer. Provided that the heavy atoms lie clo enough to the bilayer centre, they will make only a positi contribution to the Fourier transform of the unit cell. When t halogen atoms are added, all reflections which have positi signs will increase in intensity, and the intensity of all those wi negative signs will decrease. The phases of weak reflection which may pass through zero on addition of the halogen atom can be checked by an experiment in which only half the origin number of heavy atoms are introduced.[17] Thus, all the phas can be directly inferred simply from the changes in intensity th occur when the halogen atoms are added to the bilayer.

If the observed electron density profiles for the control ar heavy atom unit cells are on the same relative scale, then multiplicative scale factor K will relate this scale to the absolu electron density scale. The value of K is easily obtained from t observed difference electron density profile, as the area und this profile is simply related to the total number of halog electrons in the heavy atom unit cell. If the area under t difference profile, the mass density of the bilayer and t lamellar spacing are measured, and the contents of the unit c are known, then K can be evaluated. However, the profile c still be translated with respect to the absolute scale. Its positi on the scale can be fixed by knowing the absolute electr density of any point on the prof as the water layer) or the mean electron density. An abs ale for the bilayer is thus established.

This method of determini phases of the X-ray reflections and an absolute elec sity scale for the bilayer was tested on a lipid multilayer composed of dimyristoyl lecithin and 40% (molar) terol. High-resolution diffraction patterns were record multilayers in which all, or a fraction of, the cholesterol es had been replaced by either 26-bromocholesterol or cholesterol (see Fig. 1). All the diffraction patterns wer atively the same. They showed 12–13 orders of a lamel ng of about 50 Å and a broad, equatorially orientated b 5 Å which showed the hydrocarbon region to be in a flui When the cholesterol in the bilayers was replaced by hal cholesterol, the principal difference observed in the d pattern was a change in the relative intensities of the peaks.

The introduction of either the or iodine analogues did not affect the higher orders, ged the intensities of the first seven orders; these w signs according to whether their intensity had i (positive sign) or decreased (negative sign). The ph nments were verified by repeating the experiments with n which only half the cholesterol molecules had been e with the cholesterol analogues. The signs for all the o he control data have been independently determined by an extensive series of swelling experiments (N.P.F. and Lieb, unpublished) and are consistent with those determined here.

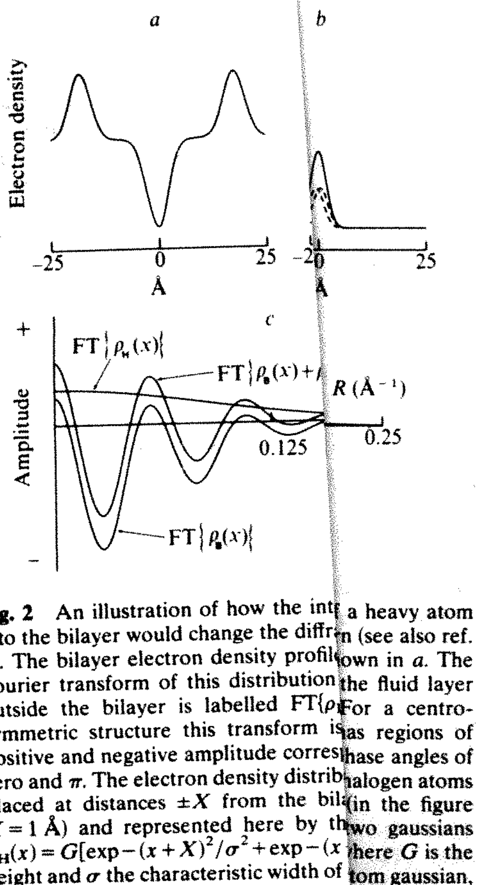

Fig. 2 An illustration of how the int a heavy atom into the bilayer would change the diffr n (see also ref. 8). The bilayer electron density profil own in *a*. The Fourier transform of this distribution the fluid layer outside the bilayer is labelled FT{ρ For a centrosymmetric structure this transform is as regions of positive and negative amplitude corres hase angles of zero and π. The electron density distrib halogen atoms placed at distances ±*X* from the bil (in the figure $X = 1$ Å) and represented here by th two gaussians $\rho_H(x) = G[\exp -(x+X)^2/\sigma^2 + \exp -(x$ here *G* is the height and σ the characteristic width of tom gaussian, is shown in *b*. The Fourier transform of ed FT{$\rho_H(x)$} in *c* and is of the form

$$FT\{\rho_H(x)\} = F(R) = 2G\sqrt{\pi}\sigma \exp -2\pi RX)$$

where *R* is the reciprocal space coordin e addition of the halogen atoms to the bilayer results i on to the unit cell Fourier transform which is a gaussia off slowly if the halogen atoms are well localised) m cosine term with a wavelength of $1/X$. The halogen at efore make a positive contribution to the unit cell tra a reciprocal space coordinate $R = 1/(4X)$, at whic cosine first changes sign and the transform becomes e figure this occurs at $R = 0.25$

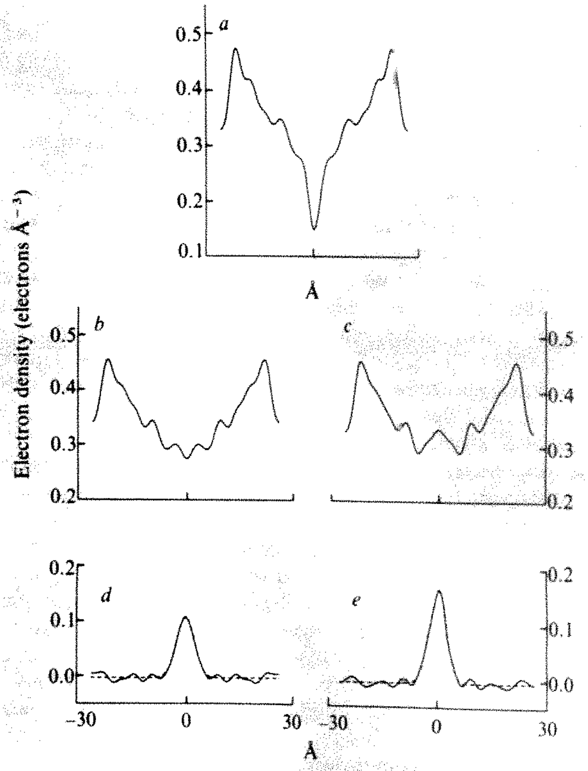

Fig. 3 Absolute electron density profiles of bilayers composed of dimyristoyl lecithin and 40% (molar) of *a*, cholesterol; *b*, 26-bromocholesterol; *c*, 26-iodocholesterol. The difference electron density profiles are shown in *d* and *e*. The profile of the bilayers containing 26-bromocholesterol is for a lamellar spacing of 52.12 Å and that of the bilayers containing 26-iodocholesterol is for a spacing of 53.20 Å. Profiles for bilayers containing normal cholesterol were calculated at both of these spacings using the swelling series data of N.P.F. and Lieb (ref. 18 and unpublished data). The determination of the absolute electron density scale is described in the text. The water contents of the multilayers were measured gravimetrically and the mass densities determined using an Anton–Parr precision density meter (we found $d_{\text{normal}} = 1.035\ \text{g cm}^{-3}$, $d_{\text{bromo}} = 1.074\ \text{g cm}^{-3}$ and $d_{\text{iodo}} = 1.090\ \text{g cm}^{-3}$). The cross-sectional areas of the unit cells (one lecithin plus 2/3 of a cholesterol) were calculated to be $A_{\text{normal}} = 67.31\ \text{Å}^2$, $A_{\text{bromo}} = 68.25\ \text{Å}^2$ and $A_{\text{iodo}} = 69.22\ \text{Å}^2$. The mean electron densities for the bilayers were 0.345, 0.355 and 0.357 electrons Å^{-3}, respectively, for the control, the bilayers containing 26-bromocholesterol and the bilayers containing 26-iodocholesterol. The assignment of phases for the first seven orders is described in the text; the phases for the higher orders were determined from swelling series and this work will be described elsewhere (N.P.F. and Lieb, in preparation). The samples were prepared on curved glass substrates from solutions of the lipids in 10:1 (v/v) chloroform/methanol as described previously[22]. The essential details of the experimental method and the correction of the diffracted intensities have been described previously[6].

The electron density profiles for lecithin/cholesterol bilayers containing normal cholesterol, 26-bromocholesterol and 26-iodocholesterol are shown in Fig. 3*a–c*. Ignoring for the moment how the absolute electron density scales in Fig. 3 were determined, consider first the general features of the profiles. The control electron density distribution of Fig. 3*a* shows a familiar bilayer profile with peaks at about ±22 Å from the bilayer centre, due to the phosphate groups of the lecithin molecules, and a well defined trough of low electron density in the centre of the bilayer where the terminal methyl groups are localised. The most striking change in the electron density profile that occurs when normal cholesterol is exchanged with either of the halogenated analogues is the large increase in electron density in the centre of the hydrocarbon region (see Fig. 3*b*,*c*). This large change is accompanied by a small, but significant, smoothing of the fine detail in the profiles, interpreted as a small increase in disorder in the bilayer due to the introduction of the halogen atoms. Note that, apart from this slight overall increase in disorder, the effects of the halogen atoms are confined to a narrow region near the bilayer centre; the appearance of the head-group region and the distance across the bilayer between the phosphate groups remain unchanged.

Small increases in cross-sectional area were observed when the halogen atoms were added. This is consistent with the increased disorder observed in the one-dimensional electron density profiles. The scale factor between the control and heavy atom profiles was refined by minimising the difference in the profiles over the head-group region. The observed increase in disorder was accounted for in the refinement by applying a imple gaussian temperature factor[19] to the control profile. Both his temperature factor and the scale factor were allowed to vary as to minimise the difference.

The difference electron density profiles are shown in Fig. 3*d* d *e*. The electron density added to the bilayer by the introction of the halogen atoms can be clearly seen to be confined region near the bilayer centre, the ripple in the background ng within experimental error. The dashed line with each rve is the predicted electron density profile for a gaussian tribution of the appropriate halogen atoms placed at mean sitions $\pm X$ from the bilayer centre. For both bromine and line, excellent agreement is obtained between the observed d predicted profiles when X is 0.8 Å and 0.2 Å, respectively, th a Debye–Waller temperature factor of 420 Å^2.

In order to put the profiles of Fig. 3 on an absolute electron ensity scale, the multiplicative factor K was first evaluated. It was found that the scale factor for the two halogens agreed to within 4%, thus giving a measure of the overall precision of the absolute scale determination. The mean of the two values was used for the scale shown in Fig. 3. Having determined the multiplicative scale factor the profiles could be fixed on the scale by using the calculated values for the mean electron densities of the bilayers. Where comparison can be made (for example, for the lecithin head-group region) the electron density scale derived here agrees well with values deduced from partial specific volume data and crystallography[20,21]. The absolute scale imposes important constraints on molecular models of these bilayers. We hope that this new method of determining phases and an absolute electron density scale will be extended and applied to both natural membranes and to protein-containing model systems.

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Role of DNA poase γ in adenovirus Dplication

THE linear DNA of th adenoviruses type 2 and type 5 (Ad2, Ad5) replicat ucleus of permissive cells by a mechanism different f of chromosomal DNA or papovavirus DNA. Papov plicate bidirectionally, with both parental strands be ated almost synchronously, whereas adenovirus DNA n occurs unidirectionally by a strand displacement m No new DNA polymerase has been observed in Ad cells[2]. To determine which cellular DNA polymera uired for adenovirus DNA replication we used the analogue 2′,3′-dideoxythymidine triphosphate (dd h inhibits DNA polymerase β, and in particular DN rase γ but not DNA polymerase α (refs 3, 4). Add TTP to isolated nuclei which synthesise adenovirus D results in a strong inhibition. In similar conditions ce or SV40 DNA synthesis is not affected. In view of e of DNA polymerase α and γ, but not β, in adenovi tion complexes[5,6] our results indicate that only DNA e γ is required for adenovirus DNA chain growth.

KB cells were infec Ad5 or Ad2 and 16 h after infection, when viral D ation is maximal, nuclei were isolated. The nuclei we d at 32 °C in the presence of the ingredients neces *vitro* DNA synthesis[7] and various concentrations ^{3}H-dGTP was used as the radioactive label and P and dTTP were present at 50 µM. After 30 min t was stopped, viral DNA was selectively extracted -dGMP incorporation was measured after precipi chloroacetic acid. The results (Fig. 1) show that ade A synthesis is very sensitive to ddTTP, with 50% i a ratio (ddTTP)/(dTTP) = 1.4×10^{-2}. Cellular D is is not inhibited up to a ratio (ddTTP)/(dTTP) = 20 dATP is used as nucleotide analogue, instead of ilar results are obtained (data not shown).

We have also stu ect of ddTTP in nuclei from primary monkey cell These cells are permissive for SV40 but are semi-p adenovirus infection, caused by a block in some without affecting viral DNA synthesis[8,9]. Hence, provide the possibility to study the effect of ddTTP ent viral systems in one type of host cell. Figure 1 s so in AGMK cells Ad5 DNA synthesis is very sens TP while SV40 DNA synthesis is only inhibited whe resent in at least 20-fold excess compared to dTTP. s the results of Edenberg *et al.*[3] for SV40 DNA syn lear lysates.

0028–0836/78/0276—0532$01.00

The inhibition of adenovirus DNA synthesis *in vitro* is independent of the time of incubation and can be suppressed by an excess of dTTP. At 500 μM dTTP, 1 μM ddTTP inhibited only 4% while at 50 μM dTTP 60% inhibition was observed, with the same concentration of ddTTP. Evidence for the competitive character of ddTTP inhibition was obtained by varying the nucleoside triphosphate concentration (S) in the presence of different ddTTP concentrations. Straight parallel lines were observed when S was plotted against S/V (not shown), giving an apparent K_m of 1.1 μM for dNTP and a K_i of 0.08 μM for ddTTP.

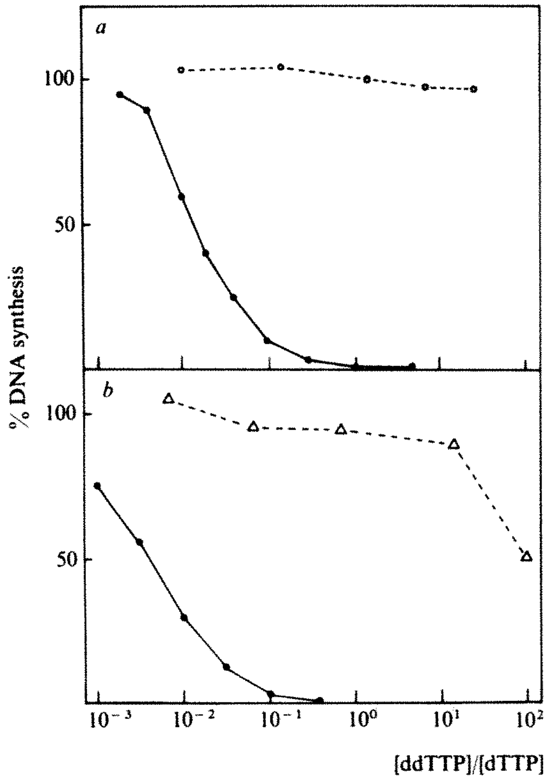

Fig. 1 Inhibition of adenovirus DNA synthesis in isolated nuclei by ddTTP. KB cells (*a*) or AGMK cells (*b*) were infected with Ad5 (○) or SV40 (△). At 16 h (Ad5) or 40 h after infection (SV40) the cells were collected and nuclei were prepared[7]. The nuclei were incubated in a final volume of 0.1 ml (2×10^7 nuclei per ml) in a reaction mixture for DNA synthesis, containing ddTTP (PL Biochemicals) as indicated. Final concentrations were 40 mM *N*-2-hydroxyethylpiperazine *N*′-2-ethane sulphonate (*p*H 7.8), 55 mM NaCl, 45 mM KCl, 5 mM $MgCl_2$, 0.4 mM $CaCl_2$, 1 mM dithiothreitol, 1 mM phosphoenol pyruvate, 2 mM ethylene-glycol tetra-acetate, 2 mM ATP, 0.05 mM each UTP, CTP, GTP, dATP, dCTP, dTTP, and 1 U ml^{-1} pyruvate kinase. ^{3}H-dGTP was present at 5 μM and 2 Ci $mmol^{-1}$. After 30 min at 32 °C the reaction was stopped by addition of 1 ml of 0.01 M Tris-HCl (*p*H 7.5), 0.01 M EDTA and cooling. To uninfected or Ad5-infected nuclei 1 ml 10% TCA containing 0.01 M sodium pyrophosphate was added. After 15 min TCA-precipitable material was collected on Whatman GF-C filters, washed with 50 ml 0.5% TCA–1 mM sodium pyrophosphate and with 96% ethanol, dried and counted in a liquid scintillation counter. From part of the nuclei, before addition of TCA, Ad5 DNA was selectively extracted[7] and the viral nature of the newly synthesised DNA was established by CsCl gradient centrifugation. SV40 DNA synthesis was determined by extraction of the DNA[17] followed by TCA precipitation. Part of the SV40 DNA was analysed by sucrose gradient velocity sedimentation in the presence of ^{32}P-labelled marker DNA. The DNA sedimented at 22–28 S with a pattern characteristic for SV40 DNA synthesised *in vitro*[18]. 100% = 20,530 c.p.m. per 10^6 nuclei (Ad5), 2,530 c.p.m. per 10^6 nuclei (uninfected) and 7,241 c.p.m. per 10^6 nuclei (SV40). ○, Uninfected KB cells.

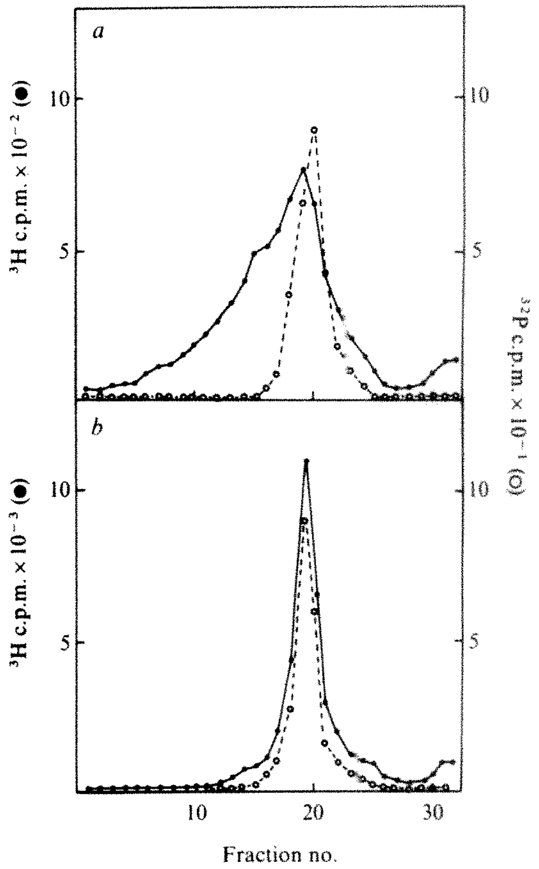

Fig. 2 Neutral sucrose gradient sedimentation of adenovirus DNA synthesised in isolated nuclei in the presence (*a*) or absence (*b*) of ddTTP. KB cells were infected with Ad5 and 16 h after infection nuclei were isolated. The nuclei were incubated as described in Fig. 1 in the presence of 2 μM ddTTP ((ddTTP)/(dTTP) $= 4\times10^{-2}$) which gives 80% inhibition compared to the control, without ddTTP. Viral DNA was extracted[7] and dialysed against 0.01 M Tris-HCl *p*H 8.0–0.01 M EDTA-1M NaCl. Samples obtained from 2×10^6 nuclei were mixed with ^{32}P-labelled Ad5 DNA and layered on a 5–27% isokinetic sucrose gradient in 0.01 M Tris-HCl *p*H 8.0–0.01 mEDTA–1 M NaCl–0.1% Sarkosyl. Centrifugation was performed for 16 h at 24,000 r.p.m. at 4 °C in a SW 41 rotor. Sedimentation is from right to left. When tested in a CsCl equilibrium centrifugation run all ^{3}H-labelled DNA had the expected density of Ad5 DNA, 1.761 g cm^{-3}.

To study the mechanism of inhibition, Ad5 DNA synthesised in the presence of 2 μM ddTTP, which gives 80% inhibition, was analysed by neutral sucrose gradient centrifugation (Fig. 2). 67% of the newly synthesised DNA sedimented between 31S and 68S, which is the sedimentation range of replicative intermediates, while in the control, without ddTTP, only 7% replicating DNA was found. In alkaline conditions, the viral DNA made in the presence of ddTTP appeared as molecules smaller than genome length (data not shown). These results can be explained by assuming that ddTMP is incorporated into growing DNA chains and acts as a chain-terminator, a mechanism that is proposed for the action of ddTTP on DNA polymerase I of *Escherichia coli*[10] and for the effect of ddTTP on ΦX174 DNA replication in permeabilised *E. coli*[11].

DNA polymerases α, β and γ from CV1 cells or HeLa cells can be distinguished by their different sensitivity to ddTTP[3,4]. We have confirmed these results with purified KB-DNA polymerases (Fig. 3). DNA polymerase α is most resistant with 50% inhibition at (ddTTP)/(dTTP) = 30. DNA polymerase β is partially resistant (50% inhibition at (ddTTP)/(dTTP) = 1) while DNA polymerase γ is most sensitive. When assayed with activated DNA in the presence of 5 mM Mg^{2+}, 50% inhibition was found at $(ddTTP)/(dTTP) = 6 \times 10^{-2}$ while in the presence of poly (rA)·oligo(dT) as template-primer and 0.5 mM Mn^{2+}, the preferred test conditions for DNA polymerase γ, the enzyme was inhibited even more markedly by the nucleoside analogue. A similar result was obtained with ddATP as inhibitor in the presence of activated DNA.

In view of the large difference in sensitivity between DNA polymerases α and γ and the specific inhibition of adenovirus DNA synthesis by ddTTP and ddATP, our results can be best explained by assuming that DNA polymerase γ is required for chain elongation of adenovirus replicative intermediates. The concentrations of ddTTP giving 50% inhibition of both adenovirus DNA synthesis and DNA polymerase γ activity are of the same order of magnitude and ddTTP behaves as a competitive inhibitor in both situations. Caution is required, however, when extending results obtained with purified enzymes to the more complicated situation that exists in the replication fork. A function of DNA polymerase β in viral DNA chain growth is less likely since only α and γ enzymes are found in replication complexes capable of elongation[5,6] and since adenovirus DNA synthesis is sensitive to low concentrations of *N*-ethylmaleimide, which do not inhibit DNA polymerase β activity.

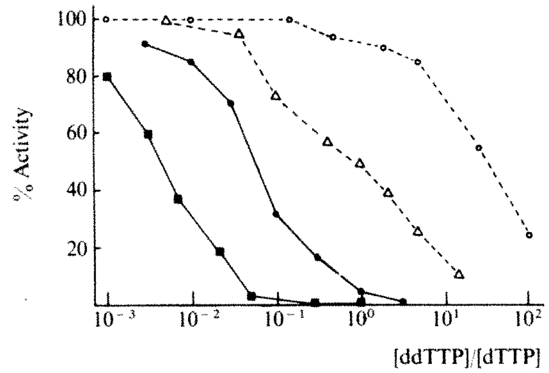

Fig. 3 Effect of ddTTP on DNA polymerase α, β and γ. DNA polymerases α and β from KB cells were purified by DEAE cellulose and DNA cellulose chromatography[2]. DNA polymerase α was characterised by sedimentation (8.8S) and its sensitivity to *N*-ethylmaleimide (95% inhibition by 1 mM NEM). DNA polymerase β sedimented at 3.4S and was completely resistant to 5 mM NEM. DNA polymerase γ was isolated according to Knopf *et al.*[19] and purified by DEAE-cellulose, phosphocellulose, hydroxylapatite and DNA cellulose chromatography. The enzyme preferred poly (rA)·oligo(dT) as template-primer to activated DNA, was 85% inhibited by 5 mM NEM and was essentially free of contaminating DNA polymerase α of β, as indicated by the absence of remaining enzyme activity at a (ddTTP) to (dTTP) ratio >1. The DNA polymerases were tested[19] with 5 mM NEM present in the β-polymerase incubation mixture. ○, DNA polymerase α; △, DNA polymerase β; ■, DNA polymerase γ with poly $(rA)-dT_{12-18}$ as primer-template; ●, DNA polymerase γ with activated DNA as primer-template.

The function of DNA polymerase γ in the cell is unknown. Recently, several laboratories[12–14] have described the identical properties of nuclear DNA polymerase γ and mitochondrial DNA polymerase. Interestingly, both mitochondrial DNA[15] and adenovirus DNA replicate unidirectionally according to a strand-displacement mechanism, in contrast to nuclear DNA synthesis or papovavirus DNA replication. Another significant difference is the absence of histones in intracellular adenovirus DNA[16]. The above differences, together with our results, indicate the presence of at least two enzymatically distinguishable replication mechanisms in the mammalian cell.

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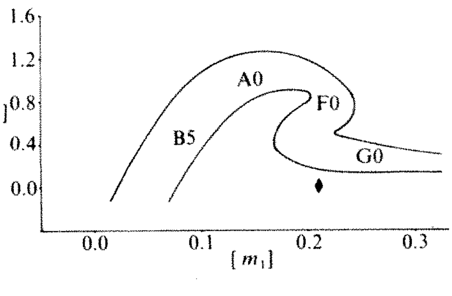
Errata

In the article 'Nucleotide sequence of bacteriophage G4 DNA' by G. N. Godson, B. G. Barrell, R. Staden and J. C. Fiddes, *Nature* **276,** 236–247, the two sentences starting on line 2 of page 242 should read: 'The amino acid differences between the proteins of the two phages is shown in Table 1. Gene *D* is the most highly conserved protein between the two phages, with only 17.8% of the amino acids changed, but this may be partly due to the presence of the overlapping *E* gene within the gene *D* coding region (see below).'

In the letter 'Are receptor-activated ciliary motor responses mediated through voltage or current?' by J. de Peyer and H. Machemer, *Nature* **276,** page 285, the last sentence in paragraph 1 should read: 'This is the first report of intracellularly recorded receptor currents in ciliates.'

In the letter 'Site of $1,25(OH)_2$-vitamin D_3 synthesis in the kidney' by M. G. Brunette *et al.*, *Nature* **276,** page 287, line 16 in paragraph 3 should read: 'were less readily digested by collagenase, and could *not* be dissected'

In the letter 'An ultraviolet subdwarf companion to HD17576' by J. Darius and P. A. Whitelock, *Nature* **275,** page 428, Fig. 2 was printed without the diamond symbol representing the position of HD17576. The figure is shown correctly below.



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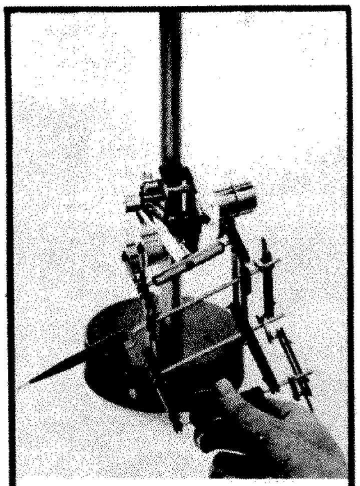

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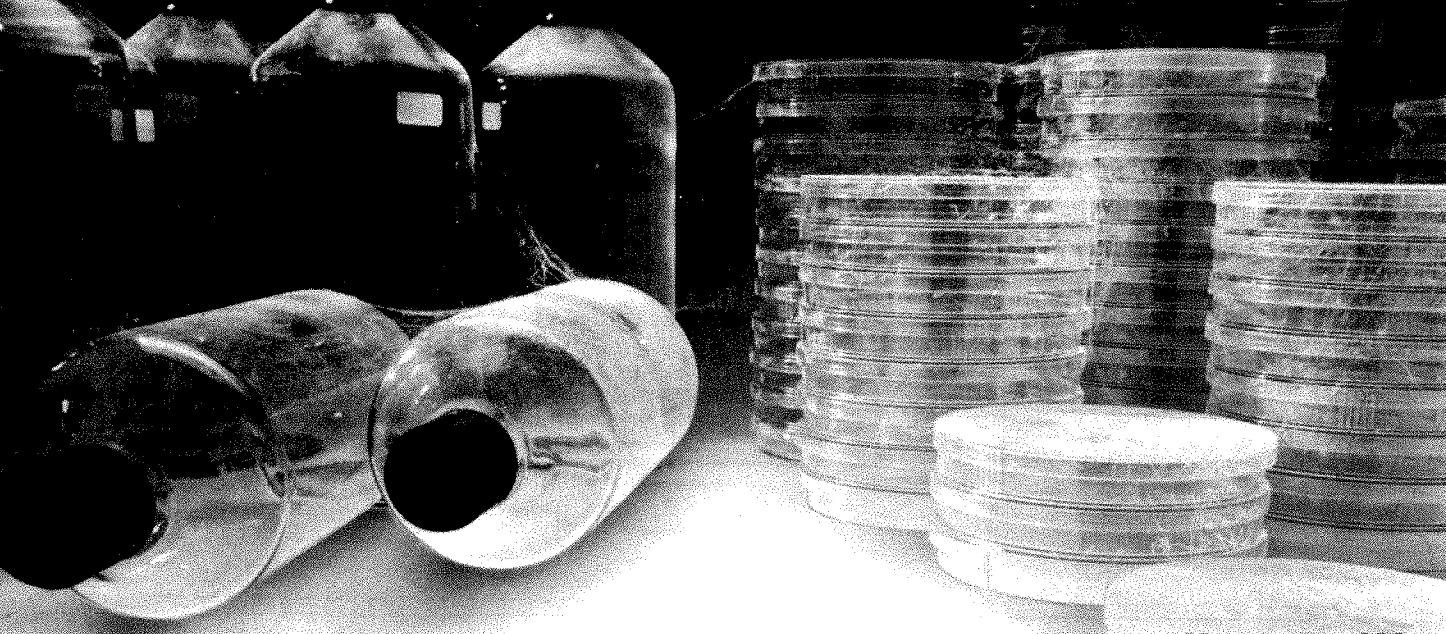

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matters arising

Large circles on the Earth's surface

AT the 3rd International Conference on Basement Tectonics in Durango on 15–19 May 1978, slides of the large circles in Arizona described by Saul[1] were shown. Several viewers pointed out that the largest circle in Fig. 1 of this paper had also been seen on satellite (Landsat) images, contrary to Saul's experience, and that such images are advantageously used in conjuction with the photographs of raised relief maps.

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1 Saul, J M *Nature* 271, 345–349 (1978).

SAUL REPLIES—Several investigators have noted that some of the strongest circles are visible on satellite photographs and that some are also discernible on geological and tectonic maps. Satellite photographs of areas that have partial snow cover seem particularly useful. At present the original photomaps made by James Lindsay seem to be the most informative. Lindsay had noted the largest circle in Fig. 1 ref. 1 several years ago as well as strong linear features, some of which have apparently not been noted on more conventional types of coverage. The appearance of these linear features is strongly dependent on the direction of lighting. Several people pointed out circular features in the Canadian Shield, South Africa, East Africa, South Australia and the Himalayan region but these were found on a great variety of maps, photographs and plots, and their identity with those produced by photographing raised relief maps was surmised rather than demonstrated. One of the most interesting was the circular pattern noted by G. A. Kellaway for tin lodes in Cornwall. S. Parker Gay, Jr has identified large circles using the techniques he has developed for viewing geophysical data and topographic information in stereoscopic pairs[2].

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1. Saul, J M *Nature* 271, 345–349 (1978)
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Lifetime of an elliptical ring around Uranus

LUCKE[1] suggests that Uranus' ε ring is composed of particles in elliptical orbits, with slightly different eccentricities and nearly perfect alignment of orbital axes. This model can fit observational data[2–4] obtained in 1977 during the occultation of the star SAO158687. We point out that the model probably works only if the ring is a short-lived configuration, because perturbations due to Uranus' oblateness quickly change the shape of the ring.

If we consider the precession period P of apsidal lines in a ring lying in the planetary equatorial plane, we have

$$P \simeq \frac{4\pi a^{7/2}(1-e^2)^2}{3J_2R^2(GM)^{1/2}} \simeq 98\text{ d} \qquad (1)$$

where M, R, J_2 are the mass, radius and form factor of Uranus respectively (this latter is not well known; we use a typical value of 0.01 (ref. 5)); a and e are the semimajor axis and eccentricity of the satellite particle. If two particles in the ε ring have semimajor axes and eccentricities differing by Δa and Δe, the precession periods differ by a quantity

$$\Delta P = \left(\frac{7\Delta a}{2a} - 4\frac{e\Delta e}{(1-e^2)}\right)P \qquad (2)$$

and a randomisation of axis direction is likely to occur in a time

$$T \simeq P^2/\Delta P = P\Big/\left(\frac{7\Delta a}{2a} - 4\frac{e\Delta e}{1-e^2}\right) \qquad (3)$$

It is unlikely that all pairs of particles have orbits with a and e differing by just the quantity needed to make the denominator of equation (3) very small. Therefore, an upper limit on T may be obtained by assuming $\Delta a \simeq 0$, $e \simeq 0.008$ and $\Delta e = 0.0005$ (the latter values are chosen to account for the width variation of the ring[1]). We get in this case $T \simeq 6.3\times10^4\,P \simeq 16{,}900$ yr. A more reliable value of T may be obtained if we consider that interparticle collisions are not likely to allow $\Delta a \simeq 0$; for $\Delta a/a \simeq 10^{-3}$ (which corresponds to the width of the ring), we get $T \simeq 300\,P \simeq 80$ yr.

A similar phenomenon occurs if the ring lies out of the equatorial plane. In this case, the precession of the line of nodes yields, in a time of the order of T, a symmetrical band (with respect to the equatorial plane) that subsequently flattens out as a result of inelastic collisions among particles[6].

We conclude that the ε ring, if composed of particles in elliptical (or inclined) orbits around Uranus, may be only a transient phenomenon, and that the ring perhaps evolves in a way which may be observed.

We thank G. Colombo for useful discussions.

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Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted Neither contribution nor reply (if one is necessary) should be longer than 300 words, and the briefest of replies, to the effect that a point is taken should be considered.

Why should the genome congeal?

A NUMBER of theoretical studies during the past decade have dealt with the problem "Why does the genome not congeal?"[1] that is, why are co-adapted gene combinations repeatedly reshuffled? As a result, specific conditions which may favour recombination have been de-

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, such conditions are
only to restricted seg-
e. Therefore, selection
ination should be the
an the rule and hence
s conclusion "such models explain why the genome does not congeal, but not why recombination is as free as it is"[9]. This discrepancy between the observed and expected recombination frequencies may stem from an oversimplification inherent in the theoretical approach; namely, the consideration that recombination within a given chromosomal region is independent of other regions. This assumption may be unfounded in view of increasing data clearly indicating the interrelation of recombination frequencies within different chromosomal regions.

A recombination control system[10] involves controlling elements (genes determining recombination frequencies) and controlled elements (chromosomal sites recognised and acted upon by the products of specific controlling genes). The recognition sites of any recombination control system are not necessarily linked to the controlling genes, but may be distributed over the entire genome. Consequently several unlinked regions may be controlled by the same gene, as demonstrated in *Schizophyllum*[11–13] and *Neurospora*[14,15]. On the other hand, a chromosomal segment flanked by any two recombining markers, A and B, may well include recognition sites pertaining to different control systems. Thus, recombination rates between two particular markers may be influenced by several different controlling genes, a situation known to exist in *Schizophyllum*[16,17], *Tribolium*[18] and *Neurospora*[14].

If the region between A and B includes recognition sites for the controlling loci C_1, C_2 and C_3, recombination between A and B will be influenced by the particular alleles at C_1, C_2 and C_3. Selection favouring recombination between A and B may lead to an increase in the allelic frequencies of high recombination alleles at all three loci. As these loci control recombination rates in additional regions as well, any increase in high recombination alleles may elevate recombination rates in regions other than A–B. Consequently, selection favouring recombination within a particular chromosomal region may result in increased recombination rates elsewhere. Therefore, high recombination frequencies between two given markers may occur as an indirect result of selection favouring recombination in another region. Theoretically, the same indirect effect should also be apparent when recombination within a given region is disadvantageous. However, since low recombination rates are directly selected for over most of the genome, the indirect effect of selection against recombination is expected to be less obvious.

Practically all investigations of naturally occurring recombination-control genes conclude that low recombination is dominant to high (as shown in *Neurospora*[15], *Tribolium*[18], *Ustilago*[19], *Drosophila*[20] and *Schizophyllum*[21]). These experimental results concur with the theoretical model[6] predicting that individual selection for recombination would favour recessive high recombination alleles.

Dominance relationships between controlling alleles are particularly relevant to two aspects of selection affecting recombination rates. First selection against a recessive trait. Selection against recombination is essentially selection against a recessive trait[15,18–21]; a process rarely culminating in fixation. Therefore, high recombination alleles would remain in the gene pool despite vigorous and long-term selection against recombination. Second, the 'hitchhiking effect': Strobeck *et al.*[5], proposing hitchhiking as the mechanism for the propagation of high recombination genes, had to assume close linkage between the controlling gene(s) and the recombining markers. This assumption considerably weakens the efficiency of selection favouring recombination. However, if high recombination is indeed recessive, this assumption is unnecessary. In that case, an individual producing recombinant gametes must be homozygous at the controlling locus (loci) and the recombinant gamete will necessarily carry the high recombination allele(s). Consequently, selection favouring recombinants will result in the increase of high recombination allele frequency, regardless of its linkage relationship with the recombining markers.

Selection affecting recombination rates is operant on various levels. The rate of recombination between A and B may be altered by allele substitution at any of the relevant controlling loci. Alternatively, recombination frequency may be affected by changes in recognition sites lying between loci A and B. Although the nature of these recognition sites is still unknown, there is evidence that genetic control of recombination requires specific combinations of nucleotide sequences on the two homologues involved[22]. A particular sequence on one chromosome only is insufficient, thereby simulating a relationship paralleling, though not identical to, recessivity. Therefore, the situation concerning recognition sites may be similar to that of controlling genes, as far as the efficiency of selection against them is concerned.

The genetic control of recombination has evolved into a system that practically ensures the almost infinite storage of a high potential of recombination. In such a system, no population can effectively eliminate the controlling and controlled elements for high recombination. Consequently, even though selection favouring recombination is weak, transient and localised, it may well balance the persistent selection pressure against recombination.

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MAYNARD SMITH REPLIES—Dr Schaap makes two points. First, as loci affecting recombination do so in more than one region, higher recombination in any one region can be increased by selection, not only on the region itself, but at many others. Hence, she suggests, the total selection for higher recombination may be stronger than one would think from simple models. This is true enough. The snag is that the same is true of selection for lower recombination. I do not understand her argument that "since low recombination rates are directly selected for over most of the genome, the indirect effect of selection against recombination is expected to be less obvious".

Her second point, that alleles for higher recombination are usually recessive, is an interesting one, because selection for higher recombination arising from 'hitchhiking' is more effective in these circumstances. This tends to confirm my conviction that hitchhiking effects are the main ones responsible for maintaining high recombination.

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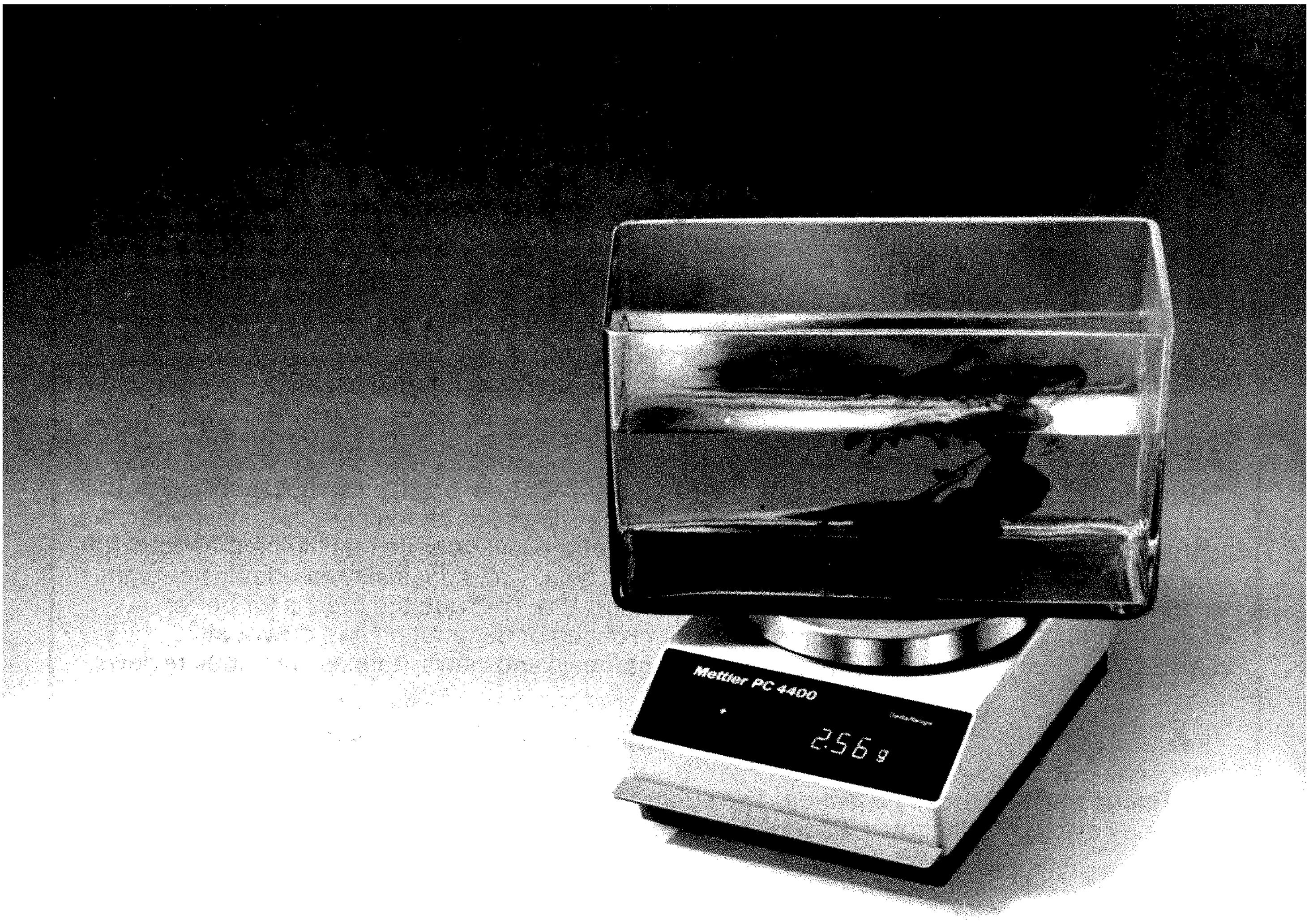

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reviews

Founders of X-ray analysis

M. F. Perutz

William Henry Bragg, 1862–1942: Man and Scientist. By G. M. Caroe. (Cambridge University Press: Cambridge and London, 1978.) £8.95.

W. H. Bragg became famous for his early work on alpha rays, for founding X-ray crystallography together with his son W. L. Bragg, and as a superb expositor of science. This book by his daughter is an affectionate memoir, vividly written, and illustrated with pictures and quotations. It has some of the lightness of touch of Gwen Raverat's *Period Piece* which conjured up the lives of the Darwins. I only just knew W. H. Bragg as a benign and dignified looking old gentleman, but I worked with his son W. L. Bragg while he was Cavendish Professor of Physics at Cambridge from 1939 to 1953 and remained associated with him until his death in 1971. Mrs Caroe's sensitive portrait told me much about her father that also illuminates the son. The book will interest many people: physicists and crystallographers, historians of science, those concerned with the social role of science, with science education, with the relationships between science and government, and between science and religion.

W. H. Bragg was the son of a merchant seaman turned farmer in West Cumberland. He won an exhibition to Trinity College, Cambridge, read mathematics and finished 3rd wrangler. One day in 1885 he walked along King's Parade with J. J. Thomson, another Trinity man with whom he often played tennis. Thomson asked whether the senior wrangler had applied for the chair in mathematics and physics at Adelaide Bragg said, "No" and wondered if he himself might stand a chance. Thomson encouraged him to apply. The appointments committee consisted of J. J., of Horace Lamb, the mathematician who had just left the chair, and of the Agent-General for South Australia. The only other candidate could not be trusted with the bottle, and so they settled for the 23-year-old Bragg who abstained from drink. Happy days!

And more to come. Young Bragg had been brought up to work and no play, and had been poor, shy and lonely at Cambridge. He blossomed out in sunny Adelaide where he enjoyed the princely salary of £800 a year and did not feel his humble origins as a social handicap. He threw his energy into work for the new University and community, married the daughter of the Government Astronomer, and became much liked and respected. The Australian setting with its pioneering enthusiasm, its outings and easygoing social life is charmingly described, as when Mrs Caroe tells us that her grandmother would try to keep her grandfather at home before parties lest he invited everyone he met in the street.

It never occurred to Bragg to do any research until, aged 42, he had to address the Australian Association for the Advancement of Science. He chose the ionisation of gases. In preparing his lecture he came across a puzzling observation by Madame Curie which showed that alpha rays were not subject to an exponential absorption law, like other radiations, but penetrated a certain distance and then suddenly stopped, like bullets shot into a piece of wood. Bragg argued that they could not be colliding with other atoms but must be passing right through them, losing energy only by the ionisation they caused on the way.

Bragg procured some radium, built an ionisation chamber and soon proved his point. He sent his first paper to Rutherford at McGill for comment and waited an interminable 3 months for a reply. When Rutherford congratulated him:

> "I think you have struck the right explanation of the absorption of alpha rays and that it is merely a matter of careful experiments along the lines you are following to disentangle it completely."

Bragg replied:

> "It is very jolly to have your comments on my paper."

This was the start of a regular and very interesting correspondence, much of which is quoted. Bragg next discovered that his preparation shot out alpha particles of four different ranges, corresponding to the four different active substances that Rutherford had shown to exist, and that the particles of shortest range came from radium itself.

Bragg then turned to gamma rays, and Mrs Caroe describes how their exploration and that of the related X-rays sent him off on a wrong tack. How could cathode rays impinging upon a target produce X-rays and these same X-rays, on hitting another target, produce secondary cathode rays? If they were electromagnetic pulses, as Stokes had suggested, then why were these secondary electrons shot out mainly in the forward direction instead of parallel to the transversely vibrating field? And why were gamma rays and X-rays themselves scattered most in the forward direction instead of uniformly in all directions?

In 1907 Bragg wrote to Rutherford:

> "I have got the decisive experimental proof that gamma rays are not pulses, but corpuscular."

In a later lecture he said:

> "The Röntgen ray must move in a straight line without loss by the way, so that when it strikes the fatal atom it may have an undiminished amount of energy to give to the electron which takes its place, and the velocity of the latter may not depend on the distance the ray has travelled from the tube . . . We must replace our spreading waves by clouds of minute bundles of energy, tiny entities which move like material particles, but with the speed of light. It is a curious return in the direction of the old Newtonian corpuscular hypothesis."

He suggests that the electron, as it is driven against the anticathode, picks up a neutralising amount of positive charge, producing a neutral pair incapable of deflection by fields.

> "The neutralising positive may be torn away again from the electron in some other transit across an atom, and the Röntgen ray became once more a cathode particle."

Rutherford was enthusiastic at first, but later wrote more cautiously:

> "I quite appreciate the utility of the physical conception in your doublet, but I would at any time be prepared to consider it a bundle of concentrated energy which has all the properties of your doublet."

Some of Bragg's difficulties would have been resolved if he had studied Einstein's 1905 paper on the photoelectric effect, but in a letter to Rutherford at the end of 1911 Bragg admits that he had not heard of Einstein when he formulated his theory. In another letter he comforts himself with what must have been a garbled message:

> "Lindemann says that Planck's theory now is that the atoms can take in energy of any wavelength *at any rate*, but can only give it out in definite amounts—relativity is rather in the background in Berlin just now."

I found this account fascinating.

In 1909 Bragg's half-dozen papers on ionising radiations brought him an FRS and the physics chair at Leeds. Happy days! the reader may think again, but in fact it was the end of the happy days. While students and public at Adelaide had lapped up Bragg's vivid lectures, they fell flat among the sober Yorkshiremen. Mrs Bragg could not get used to the dirt, the smoky dark, the poverty and the rickety children of Leeds, and Bragg's neutral pair theory ran into stormy seas. Yet amidst the acrimonious controversy with Barcla his greatness shows. He does not withdraw into cynicism, but writes to Rutherford:

> "Supposing the identity of X-rays and light to be established, the supposition is this, I take it: The energy travels from point to point like a corpuscle: the disposition of the lines of travel is governed by wave theory. Seems pretty hard to explain: but that is surely, how it stands at present."

Mrs Caroe's quotations from Bragg's early papers and lectures already show his talent for expressing science in lucid, homely language free from jargon.

Meanwhile Bragg's son, William Lawrence, having graduated in mathematics at Adelaide at 18, became an undergraduate once more at Cambridge, reading mathematics and physics. He writes:

> "Dear Dad, I'm so glad you liked the notes on Jeans. I got an awful lot from a Dane who had seen me asking Jeans questions. He was awfully sound, and most interesting, his name was Böhr or something that sounds like it."

At Leeds in the summer holidays of 1912 he found his father intensely excited about the paper by von Laue, Friedrich and Knipping on the diffraction of X-rays by crystals. Back in Cambridge, he writes to his father:

> "I want to have a good go at this crystal divining . . . But don't let on for a bit till I can try the crystal Pope (the Professor of Chemistry) is getting for me."

Later:

> "I have just got a lovely series of reflections of the rays in mica plates with only a few minutes' exposure! Huge joy."

And the father writes:

> "My dear Rutherford, my boy has been getting beautiful X-ray reflections from mica sheet just as simple as the reflections of light in a mirror,"

but in Cambridge the son was teased for having disproved his father's corpuscular theory of X-rays. The discovery of Bragg's law, the interpretation of the Germans' diffraction patterns and the determination of the structure of common salt were all done by the son alone at Cambridge.

In Leeds meanwhile the father put his experience with ionisation chambers to good use in constructing an X-ray spectrometer, with which father and son solved the structure of diamond and other simple crystals. Together they founded the science of X-ray analysis; the world reacted thoughtlessly, as though the decisive advance had been made by the father, helped along by the son. The father made matters worse by referring in a literally patronising way, to his "boy's" work, when the "boy" was craving for recognition as an independent scientist. So their great discoveries, which brought them the Nobel Prize for Physics in 1915, tragically strained relationships between them for the rest of their lives. In his many lectures on the development of X-ray analysis W. L. Bragg was fond of defining the exact roles played by himself and his father, but he never hinted at those strains until a few days before his death when he wrote to me:

> "I hope that there are many things your son is tremendously good at which you can't do at all, because that is the best foundation for a father–son relationship"

—a reflection on the painful competition between himself and his father. Mrs Caroe alludes to the conflicts between them with affection and sympathy for both.

W. L. Bragg with his father, shortly before the latter's death in 1942

In 1923 Bragg father became Director of the Royal Institution where he remained until his death 19 years later. Here he founded a flourishing school of X-ray crystallography from which much of today's biological structure analysis is descended. He also found scope for his superb gifts of popularising science, especially to children, and became a protagonist of the role that science ought to play in education, government and industry. He wanted children to learn more science, but opposed a purely scientific education that turns out backroom boys.

> "'If research workers' education were made more general, they would move into the Councils of industry to which specialists in science are as yet rarely admitted. The scientific expert must himself take down the barricade that makes the alley blind."

To this end he admonished scientists to make themselves understood:

> "We cannot compel men to make use of science in the right way, but the chance that good use will be made is in a curious way dependent on the ease with which it is stated."

Unlike Monod in *Chance and Necessity*, who tries to base a new ethics on scientific truth, Bragg thought science was neutral:

> "Science is merely knowledge. It has no morality at all. The achievements of scientists have value of one kind, and the spirit in which they worked has value of another kind. Faraday's reverence for truth and unselfish devotion to its acquisition have a higher value than the laws which he established."

Perhaps this is what Monod meant. Bragg regarded science as an ever receding frontier.

> "There is a hesitation which would beg us not to push forward lest we come to think less of the world. As against this, research is an act of faith in the immensity of things. There is no end to the search: it is a poor thought that there might be." "The work of discovery will go on and no one can stop it."

Who would dare to say this today?

In his last year at school Bragg went through a harrowing religious experience, reminiscent of the one described so movingly in James Joyce's *Portrait of the Artist as a Young Man.* A less level-headed person might have reacted by turning against religion, but Bragg tried to bring religion into harmony with science, because he had faith in both,

> "From religion comes a man's purpose; from science his power to achieve it. Sometimes people ask if science and religion are not opposed to one another. They are: in the sense that the thumb and fingers of my hand are opposed to one another. It is an opposition by means of which anything can be grasped."

His daughter writes of him:

> "Religious faith to him was the willingness to stake his all on the hypothesis that Christ was right and test it by a lifetime's experiment in charity."

His charity found expression in the help he gave to Jewish scientists when they were driven from Europe by the Nazis, and again when they were misguidedly interned here as potential spies during the Second World War. In general, Bragg spoke only of the benefits that science would bring, but once he wrote:

> "If it falls into the hands of evil persons working for evil ends we, as scientists, can do nothing about it."

I am surprised that he should have taken that easy line, perhaps because he was spared the heart-searching in the aftermath of Hiroshima.

Rutherford, another farmer's son, regarded Bragg "as a very great man", but Bragg himself remained diffident about the qualities that had raised him to the Presidency of the Royal Society. One evening, opening his study door, his daughter noticed how wearily he looked up from his papers.

> "Daddy, need you work so hard?"

she cried, and he answered simply,

> "I must dear; I'm always afraid they'll find out how little I know."

Bragg's last scientific function was a radio discussion with Bernal on living

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crystals. It was all about the nature of proteins and viruses. Bragg:

> "How do protein molecules get folded? Can no one make a protein?"

Bernal:

> "It looks as if all proteins are made by others inside the cells."

Bragg:

> "But if one protein must always come from another, how did the first one get there?"

Bernal's answer avoids this paradox.

> "One big step towards finding out is getting the structure of proteins and viruses It's really the problem of the origin of life."

No inkling yet about the role of nucleic acids. Bernal had been Bragg's research student, and Bragg may have had him in mind when he wrote:

> "A good research student is like a fire which needs but a match to start it."

Quotations such as this one make the book delightful reading.

I was intrigued by the similarities between father and son. They had both read mathematics, but neither was a theoretician at heart, and they solved scientific puzzles in a concrete, visual fashion. The father complained of Planck not bothering to think about the physical nature of the quantum and to the son quarks had no appeal. To them a structure solved was like an artist's vision, to be described in words of poetic imagery. They felt more at ease with children than adults and preferred to deal with things rather than people. They were no match for politicians: the father was taken for a ride by the higher civil servants when he demanded that science should have a stronger voice in the conduct of the war; the son was helpless among the Byzantine complexities of University politics. The father's faith in God and the goodness of science marks him out as a man of the last century rather than of this one; the son avoided ultimate questions and tended his garden.

What would the reader gain from this book? Much enjoyment; the realisation that success in science can be combined with devotion to human values, and that occasionally the great can also be good and true ☐

M. F. Perutz is Chairman of the Governing Board of the MRC Laboratory of Molecular Biology, Cambridge, UK

DNA inconstancy

Endopolyploidy and Polyteny in Differentiation and Evolution. By W. Nagl. Pp. 283 (Elsevier/North-Holland: Amsterdam, New York and Oxford, 1978) $54 50; Dfl.125.

As indicated by its title, this book is concerned with the relationships between two of the central issues in biology—namely, differentiation and evolution The thread that connects the two, as seen by the author, is the occurrence of DNA variation as an important aspect of both Evolution has been accompanied by remarkable shifts in the amount, and in the organisation, of basic genomal DNA in different species Moreover, in very many Orders of animals and plants, there occurs varying degrees and types of ontogenetic elaboration of the basic genome These are seen in their most obvious expression as polyteny and endopolyploidy, and more subtly, as amplification of certain selected regions of the genome such as the ribosomal cistrons.

To many biologists, such expressions of DNA inconstancy are mere curiosities which, though useful experimentally, serve to emphasise the rule of DNA constancy, with normal mitosis as the orthodox mechanism of DNA growth. Reading this book will do much to redress the balance for, far from being the exception and the concern of insects and bugs, DNA inconstancy is to be found universally In fact, after reading this book one feels that the cell nucleus has such a penchant for ontogenetic elaboration that the problem becomes not how or why this occurs, which are matters discussed at length, but how species manage at all to maintain DNA constancy for the purposes of ensuring their continuation.

In all probability it is precisely the failure of species entirely to constrain the exuberance of DNA in this respect that is one of the main driving forces of the evolutionary process In a very real sense, therefore, the study of ontogenetic DNA variation contains valuable clues to the understanding of phylogenesis and evolution. In other words, speciation and differentiation are two sides of the same coin, which is DNA variation Along the way to making this point the book commences with a concise and up-to-date account of the organisation of the eukaryotic chromosome and continues with chapters dealing with the occurrence and characterisation of endopolyploid nuclei, the mechanism of endopolyploidisation, further mechanisms of DNA variation, control of endopolyploidisation and polytenisation, functional significance of endocycles, and, finally, evolutionary aspects.

This final chapter brings together the threads runing through the previous parts of the book in a far ranging discussion concerned with the meaning of DNA variation in evolution. Commencing with a tabulation of various mechanisms of, and the terminology for, DNA variation in phylogeny and ontogeny, the author discusses the role of point mutations (adaptation and anagenesis) and of repetitive DNA (speciation and cladogenesis) in the evolutionary process In particular he emphasises the nucleotypic effects of alterations in DNA and of genomal DNA repatterning as important mechanisms. Nucleotypic effects are considered to be explicable principally in terms of the Britten–Davidson model of gene regulation and the relationship of DNA interspersion patterns to genome size is discussed as a possible nucleotypic effect of DNA mass Endopolyploidisation and differential DNA replications are considered as evolutionary strategies in light of the fact that, in general, these phenomena achieve their most extreme expression in species with relatively low DNA C values.

The final paragraphs of this chapter set out the author's views concerning the integration of DNA variations during ontogenesis and evolution summarised by what he terms the DNA optimisation model. This illustrates some possible changes in genomal size and organisation during phylogenesis and considers their possible consequences on development This is altogether a very thought-provoking section of the book which is otherwise an extensive and important review.

Altogether there are 283 pages, of which 49 pages are of references, including the Russian and older German papers amounting in all to over 1200 references of which 55 are to the work of the author and his colleagues These are extensively cited and critically discussed and cross-referenced, making this not a book to be read without effort. It is well and profusely illustrated with line diagrams and half-tones, and contains many unique and informative tabulations of data collected from the numerous sources cited. There is a useful subject index and two appendices containing a glossary of terms and further guidance to the methodological literature dealing with the detection of endopolyploidy and differential DNA replication, the preparation and analysis of chromosomes, *in situ* hybridisation, DNA characterisation, and genome organisation analysis

An indispensible book for the expert but aimed also at a wider readership, it should appeal to students of cytogenetics and molecular biology.

K. W. Jones

K W Jones is Reader in the Institute of Animal Genetics, University of Edinburgh, UK.

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and Recognition. Series A. Vol. 5. Edited by P. Cuatrecasas and M. F. Greaves. Pp. 212. (Chapman and Hall: London, 1978.) Hardback £10; paperback £6.

PREVIOUS volumes in this series have contained four or five articles, but this one contains only three, albeit rather longer, contributions. "Relationships in the structure and function of receptors for glycoprotein hormones, bacterial toxins and interferon" by L. D. Kohn is a contribution central to the theme of the series, whereas "Stereoselective recognition in biology" by P. A. Lehmann and "Fluorescence and NMR studies of membranes" by A. G. Lee can be considered to some degree as contributions to the general education of those interested in biological recognition processes.

Lehmann's chapter is a wide-ranging discussion of the ways in which the mutual recognition between macromolecules and small ligands may be influenced by stereochemical variations in the nature of the bound ligand. It draws its examples from an extraordinary diversity of receptor and enzymic recognition systems, thus placing shoulder to shoulder the pheromones of bark beetles, the proteolytic specificity of papain, conformationally hindered thyroxine derivatives and the interaction between avidin and biotin. Not unnaturally, substantial space is given to the author's own 'eudismic' method for deriving quantitative relationships between affinity and stereoselectivity in various series of homologous pairs of stereoisomers

As there have recently been a considerable number of reviews of the contributions of spectroscopic techniques to understanding of membrane structure, some of them by Lee himself, a chapter on this topic would only seem justified if it emphasised the ways in which such techniques might be helpful to students of biological recognition phenomena. Unfortunately, this is not what is offered. Instead, the chapter opens with good intentions of interpreting NMR data for the uninitiated, but it soon becomes yet another detailed discussion of the spectroscopic characteristics of hydrated lipids, especially dipalmitoylphosphatidylcholine. Proteins, and even receptors, do get briefly discussed in the final section on fluorescence studies, but it seems doubtful whether many general readers will get this far.

Finally, Kohn's contribution takes us to the heart of recent studies on the receptors for a superficially diverse 'family' of ligands (glycoprotein hormones, bacterial toxins and interferon). It is mainly through the work of Kohn and his colleagues, particularly their studies on gangliosides as receptor components and on sequence homology, that these ligands and their receptors can in any sense be regarded as a family. Because of this, the chapter is to be welcomed, but with the slight suspicion that Kohn may, at least sometimes, be adept at seeing unities where none exist. As this is very much the chapter of an enthusiast, it is filled with intriguing snippets of unexplained new information which will keep the reader's appetite whetted for the next episodes in this intriguing tale.

Overall, therefore, this is an uneven book with too much space devoted to material far from the nitty-gritty of receptors and recognition. Its proofreading may be a bit better than in earlier volumes (see review in *Nature* **274**, 192–193; 1978) but there is still no index (purchasers of volume 6, as yet unpublished, are to get the indices of volumes 1–5 as a bonus). **R. H. Michell**

R. H. Michell is Lecturer in Biochemistry at the University of Birmingham, UK.

Conformational energy surfaces

Biomolecular Information Theory: Studies in Physical and Theoretical Chemistry. By S. Fraga, K. M. S. Saxena and M. Torres. Pp. 271. (Elsevier: Amsterdam, Oxford and New York, 1978.) $48.75; Dfl.112.

READING this book was a horrible experience. I hasten to add that this was not a fault of the book itself, which has many commendable features. It was merely a manifestation of that recurrent nightmare of text-book writers, a new book with similarities to that which he is in the throws of writing. After some deliberation, it seemed that it would be fun to review a book from an unusually informed viewpoint, but the reader of this review is forewarned that the reviewer may be revealing some of the prejudices of a literary cuckold.

Inevitably, closer inspection revealed many important differences. It is primarily an essay in quantum biochemistry, with emphasis on the conformational energy surfaces of units of protein and nucleic acid molecules. The title is, perhaps, somewhat misleading, as the book does not contain much more about biomolecular information than most biochemical and biophysical textbooks, and there is very little information theory proper. Nonetheless, the first major section provides an illuminating discussion of topics peripheral to an information theory of biological molecules, including the mathematics of the prebiotic origins of self-replicating systems. There is also an entertaining account of the formation of amino acids in the prebiotic soup, and of the occurrence of amino acids in meteorites and Moon dust.

The second major section is largely a vehicle for discussing the stereochemical code of proteins and nucleic acids in terms of the conformational behaviour of low molecular weight analogues of their units In particular, N-acetyl N′-methylamide derivatives of amino acids are considered and there is an exhaustive treatment of their conformational energy surfaces as a function of the two major angles of rotation in the backbone. There is also an extensive discussion of the theory and methods (with appendices) for calculating such energy surfaces, and of techniques (such as minimisation and Monte Carlo) for treating more complex energy surfaces. Unfortunately, the authors run the risk of placing too much emphasis on one type of energy calculation, the semi-empirical quantum mechanical PCILO technique of Maigret and Pullman. Indeed, much of the book reads like an encyclopaedia of PCILO results. This gives the text a certain consistency, but the danger of emphasising one type of calculation is that the future usefulness of the book depends to some extent on the validity of that calculation. It was a little one-sided of the authors to opt for PCILO, because the energy surfaces calculated by this method are somewhat anomalous with respect to those derived by the classical studies, as well as by recent empirical crystal-derived potentials and extended basis set *ab initio* calculations. Some CNDO results are reported, although this is one of the few types of calculation which produces similar energy surfaces to PCILO. The consequence of this is that the stereochemical code as viewed by the authors well allows some conformational features not believed by many workers.

Incidentally, the energy maps are presented in the old angle convention, which might cause some confusion in the partially initiated, particularly as the old convention is not used consistently throughout the book.

More generally, the book is pleasant to read, although the jargon is not well suited to biologists. Its mood is technical rather than journalistic ("Valinomycin is a cyclodecadepsipeptide") A few sections, such as that on "Topological Characteristics of Energy Hypersurfaces", turned out to be more trivial than suggested by a first read.

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Some of the mathematical treatments also seemed complicated even to workers in this laboratory who had previously translated equivalent treatments into working computer programmes, although it must be admitted that there are many books which are much worse in this respect. The book seems rightly aimed at theoretical chemists, intending to introduce them to the biological applications and significance of their work.

In the last analysis, it is a book I would be glad to own.

Barry Robson

Barry Robson is Lecturer in Biochemistry at the University of Manchester, UK.

Electrical properties of biological cells

Impedance Measurements in Biological Cells. By O. F. Schanne and E. R. P.-Ceretti. Pp. 430. (Wiley-Interscience: New York and Chichester, UK, 1978.) £21.20.

THERE exist two major schools of thought about the measurement of the electrical properties of biological cells One owes its origin to a physicomathematical approach using the concept of impedance. Typically, this involves investigating the response of the cell to a wide range of frequencies followed by an attempt to model the response physically with an electrical equivalent circuit. The second school has typically been more interested in measuring the membrane resistance and in relating this to the physicochemical mechanisms determining the membrane permeability to ions. This has usually involved measuring the response to step changes of potential or current. Of course, the two approaches are complementary but it is nevertheless true to say that in the past the two schools have sometimes been far apart; even when they have come together on the same problem the interpretations have retained characteristic differences. I myself think that this is exemplified by the major difference between the approaches of Cole and of Hodgkin and Huxley to the analysis of the squid nerve fibre. The same properties of the cell lead Cole to represent the cell as behaving like an inductance, whereas Hodgkin and Huxley represented the inductance by a time dependent permeability change A trivial difference in one sense, as the representations are equivalent. A major difference from an heuristic viewpoint since one's choice of representation does determine what one thinks is worth doing in future experimentation.

The authors of this book are well aware of this difference of approach; indeed they refer to the need to "show that the results obtained by the two groups complement each other". The book begins with a valuable treatment of the experimental and theoretical methods employed. This is followed by sections on impedance measurements in nerves, in muscles, in epithelia and on cell suspensions. In the process, a vast amount of an even vaster literature is reviewed. My major criticism of the book is that the attempt to show that the results obtained by the two groups complement each other is not very well sustained. Many parts of the later chapters are simply reviews written largely in terms of the outlook of the original authors. My second criticism is that this review of the literature does not extend much beyond 1972 in many chapters.

Anyone who has written a book of this size and extent will readily understand the problems but, whether these are primarily those of the authors or publishers, the reader should be warned that he cannot expect the treatment of certain areas to be very up-to-date. There are many examples of this. Thus, in the section on passive properties of cardiac muscle, the important work of Mobley and Page in 1972 is included, but the equally important contribution of Hellam and Studt in 1974 is omitted. In the section on skeletal muscle, Costantin's work up to 1970 is included but not his 1973 work with Taylor, nor his valuable 1975 review. I cannot therefore imagine that many private buyers will find the book sufficiently useful to justify buying it, but as a library volume for reference purposes, it may still be worth considering.

Denis Noble

Denis Noble is a Fellow of Balliol College, Oxford, UK.

Techniques of centrifugation

Centrifugal Separations in Molecular and Cell Biology. Edited by G. D. Birnie and D. Rickwood. Pp.327. (Butterworths: London and Boston, 1978.) £14; $28

THIS book is concerned mainly with work that is done using preparative rotors. It proceeds from the theoretical background to consider in turn rate-zonal centrifugation, separations in zonal rotors, and isopycnic centrifugation.

The main chapter on theory acknowledges the antipathy of some biologists towards mathematics by relegating some sections to an appendix. In other chapters considerable use is made of simplified equations to illustrate principles, and even those who have not followed the derivations are encouraged to avail themselves of the resultant expressions much as they might do the time shown on an electronic watch. The design of their experiments, often an art as much as a science, may thereby be improved. Those practitioners who feel, for financial or other reasons, that their machines ought to have a shorter working week and higher payload, may benefit substantially from such considerations. Theory properly applied enables extra information, such as sedimentation coefficients, to be obtained from preparative experiments, but the authors wisely include a caution about the importance and sometimes difficulty of maintaining adequate temperature control.

The chapter on isopycnic systems emphasises the advantages of fixed angle rotors. Although it mentions lipoproteins specifically, it fails to draw attention to the satisfactory separations of glycoproteins that have been achieved with this technique. Ancillary equipment receives some attention, but the survey of types of gradient-forming devices does not mention the versatile apparatus of Hegenauer *et al.* The percipient reader will notice a few blemishes, but these are rarely misleading. In chapter 2, η is used in the conventional way for viscosity, but in later chapters symbolises refractive index. Equations 6 7 and 6.8 were first derived by Ifft *et al.* in 1961, not 1970. In chapter 8 in several places the "e" is omitted from "Weiss". The most intriguing observation is "esosteric" on p189, a term that invites adoption by anyone who can muster a credible claim. Though the chapter on analytical ultracentrifugation is strictly within the scope of the title of the book, readers interested in these matters will probably seek a more specialised text. Where repetition occurs, its objective seems to be to make each chapter fairly self-contained.

This volume is well-written, is full of practical tips, and has concentrated from diverse sources a collection of tables on the characteristics of many types of biological particles, macromolecules, gradient materials, rotors and tubes. It will afford something of value to even the most experienced worker.

P. A. Charlwood

P. A. Charlwood is on the scientific staff of the MRC at the National Institute for Medical Research, London, UK.

Nutritional problems of the Third World

Development, Reform, and Malnutrition in Chile. By P. Hakim and G. Solimano. Pp. 91. (MIT Press: Cambridge, Massachusetts, and London, 1978.) $10; £7.

THERE is a real need for examples of national programmes that actually work within the economic restraints of developing countries Development should benefit people, and the best measure of an improved quality of life is the elimination of malnutrition, which results pimarily from poverty and ignorance. It is not enough to increase food production when people cannot buy it, nor to raise incomes when people do not know what to buy. The Chilean example has much to commend it, as an attempt was made to reduce malnutrition at a time of social change The Allende régime lasted three short years, but during this period it attempted fundamental changes in economic policy by bringing large sections of industry under state ownership and extending land reform. A general redistribution of income was achieved, unemployment was reduced, and the social services were made more available to the low income groups. Food consumption increased by about 10%, although largely as a result of food imports, and the distribution of free milk by the Government was trebled In other words all the elements for the successful improvement of nutritional status appeared to be present. The question is, did it happen?

The authors of this book are frankly pessimistic, although a proper evaluation was not carried out. The free milk scheme, the major topic of their book, provided distribution from schools and clinics which were not available to all the population thereby giving preference to the richer urban community: the milk reached only 70% of the eligible beneficiaries. Although it was provided to the most vulnerable age groups, it was often shared within the family and occasionally sold. Nevertheless, 40,000 tonnes of dried milk was distributed annually. A careful analysis of Chile's nutritional problems reveals, however, that they are due to a low consumption of food rather than a lack of particular nutrients and the emphasis on protein should be challenged.

It is self-evident that the primary need of starving children is food In retrospect, the authors recognise the lack of scientific rationale for the milk programme and admit that the present oppressive government, which is now distributing 25,000 tonnes but with added fat, might be better advised. The promotion of protein-rich foods derives from old-fashioned nutritonal ideas which were outmoded at the time of Allende, and that of free milk from socio-political targets which were unrealistic for the economy. All of this is examined in detail in the book.

It could be argued that the period was too short to expect any change in nutritional status, but this is not so. The growth rate of deprived children shows a marked improvement when they are well fed: a nutritional programme in Korea has led over seven years to a national increase of 3 cm in average height and 1 kg in average weight of children Also, in Ethiopia an applied nutritional programme halved the infant mortality rate in three years. Simple statistics of this kind are essential for evaluation—relatively easy to obtain, but not apparently available from Chile over the relevant period. Such data may not be as easy to interpret as the results of laboratory experiments where everything is controlled, but it is more satisfying to try to explain successes than failures.

The authors are well aware of the economic, educational and medical factors influencing nutritional status and suggest that all were improving during Allende's time but the reader, may not be entirely convinced. In particular, although they distinguish between preventative and curative medicine, they do not stress the difference between the strictly medical and public health engineering, for example, water and sanitation The latter is probably more effective than vaccination programmes albeit much more expensive. Similarly the educational programmes were largely through the mass media and possibly did not reach or convince the most socially deprived. Finally, action to redistribute wealth which satisfied the intellectuals, may not have been as effective as claimed. But certainly the position has deteriorated since 1973 and the brave efforts of Allende reported here are worthy of this objective documentation.

The book will interest those concerned with recent Chilean history, but will be most valuable to those grappling with nutritional problems of the Third World.

D. S. Miller

D S. Miller is a Research Fellow in the Department of Nutrition at Queen Elizabeth College, University of London, UK.

Chemical and enzymic catalysis

Transition States of Biochemical Processes. Edited by R. D. Gandour and R L Schowen. Pp.616. (Plenum: New York and London, 1978.)

THE theory of absolute reaction rates, commonly known as transition state theory has been perhaps the most influential principle in the development of our knowledge of reaction mechanisms in solution. This book consists of a series of chapters written by 21 authors on the theories and methods used to investigate chemical and enzymic catalysis

The book is written in four parts. In part I the assumptions in transition state theory are examined and its application to chemical kinetics and mechanisms discussed. When enzyme-catalysed reactions are considered, the fundamentalist view is adopted, namely that catalysis originates from stabilisation of the transition state through binding to the catalyst.

In part II, which is the longest section of the volume, the main techniques used to investigate transition state structure and properties are introduced. Rightly this section is dominated by the application of isotope effects. Primary, secondary and solvent isotope effects of hydrogen are discussed as well as the heavy-atom isotope effects on carbon, nitrogen, oxygen and chlorine The first chapter of this section, however, introduces the quantum mechanical procedures which have been used to investigate enzymic transition states and reaction pathways. The last two chapters review the application of magnetic resonance techniques to investigate enzyme–ligand complexes and the mapping of reaction pathways from X-ray crytallographic data

In part III, well studied reactions are discussed which are biochemically important These include acyl, methyl, and phosphoryl transfer, acetal hydrolysis, decarboxylations and relevant models of enzymic reactions In the final part, the use of inhibitors as tools to elucidate the sources of enzymic catalysis are discussed and the application of transition state information to the design of potent drugs is described.

Thus the book ranges over all the major disciplines which have contributed to the investigation of chemical and enzymic mechanisms The editors have selected an excellent array of authors and have welded their contributions together into a text which will be of value to a wide range of chemists and biochemists.

G. Lowe

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Aims and Scope

The journal will be primarily concerned with the publication of ethological and sociobiological data and theories presented as descriptive studies, experiments, literature reviews, or in papers dealing with theory development and refinement. As the primary focus of the journal is on the human species, the aim is to foster the dissemination of scientific knowledge in ethology, sociobiology and closely related fields, particularly as this knowledge applies to humans. Findings and theories largely concerned with nonhuman species will be acceptable to the journal provided the human implications are specified.

Forthcoming Papers in 1979

Kin Selection and Exchange in a Hunter-Gatherer Society
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Long Distance Transmission of Facial Affect Signals
Joseph C. Hager and *Paul Ekman*
Selfishness, Altruism, and True Altruism: A Proposed Taxonomy of Aid-Giving Behavior
David P. Barash
Who Looks at a Baby in Public?
Christine L. Robinson, Joan S. Lockard and *Robert M. Adams*
On the Isomorphic Structure of Endings
Robert D. Deutsch

1979 Subscription Information

Ethology and Sociobiology will commence with Volume I in January, 1979, publishing 4 issues through the year. The subscription rate is $55.00 including postage and handling for institutions, and $27.50 including postage and handling for individuals. Individuals must send remittance with their orders. (Subscribers outside the U.S.A. should add the following amounts: surface mail: $4.00; air mail to Europe and Canada: $10.00; air mail to the rest of the world: $16.00.)

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ES HOUCHES Summer School 1979

2–28 July

PHYSICAL COSMOLOGY

Physics and evolution of the universe as a whole

Cosmological models confronted with observations — J. LEQUEUX — *Observatoire de Paris - Meudon.*

The early universe. I. D. NOVIKOV, *Academy of Sciences, University of Moscow.*

Formation and evolution of galaxies and clusters of galaxies — J. E. GUNN, *California Institute of Technology.*

Masses of galaxies and clusters of galaxies — P. J. E. PEEBLES, *Princeton.*

The observational cosmological parameters — G. TAMMANN, *Université de Bâle.*

Quasistella objects; radiogalaxies and interstella matter — M. REES, *Institute of Astronomy, Cambridge* and M. LONGAIR, *Mullard Observatory, Cambridge.*

There will be an additional programme of seminars on theoretical and observational topics. The lectures will start from a basic level and will progress to the recent advances in physical cosmology. Emphasis will be put on the observational and physical grounds of cosmology.

Astrophysicists interested in observational or theoretical cosmology, as well as physicists working on related topics are welcome to apply. About 50 participants will be chosen by a committee meeting in early March 1979. Scientific organisers are J. Audouze and D. N. Schramm.

30 July–30 August

BIOPHYSICS of MEMBRANES and INTERCELLULAR COMMUNICATION

This session will review the present knowledge on the molecular organisation of the cell membrane and the mechanisms of intercellular recognition and information transfer at the extracellular level. The analysis in terms of molecular structure and physico-chemical interaction will be emphasised. Particular attention will be paid to the common molecular aspects of the various biological fields concerned. Extended and progressive series of lectures will cover:

The cell membrane — S. J. SINGER, La Jolla.
Structure and dynamics of membrane components: Proteins — M. KARPLUS, M.I.T. and R. HENDERSON, M.R.C. Cambridge. Lipids — J. SEELIG, Basel. Carbohydrates — N. SHARON, Rehovoth.
Lateral motion in membranes — H. M. McCONNELL, Stanford.
Channels and ionophores in artificial membranes — P. LAUCER, Konstanz.
Excitable membranes — C. BERGMAN, Paris and H. MEVES, Plymouth.
Synaptic transmission — P. ASCHER, Paris and J. P. CHANGEUX, Paris.
Hormonal receptors and intramembranous transduction — S. JARD, Paris and M. RODBELL, Bethesda.
Immunological recognition — M FOUGEREAU, Marseille and J. F. A. P. MILLER, Melbourne.

Other lecturers will give short courses on related topics. Scientific organisers are Marc Chabre, Grenoble, and Philippe F. Devaux, Paris.

The school is intended for young biologists involved in one of the domains treated, and willing to broaden their knowledge and to study the more fundamental aspects; they should have a good understanding of modern physical and chemical methods. The courses will also interest physicists or chemists wishing to orient towards this type of biological problems, and possessing already a basic knowledge of molecular biology and biochemistry.

Established in 1951, the school occupies a group of mountain chalets surrounded by meadows and woods, in the French Alps near Chamonix and facing Mont Blanc. The location offers mountain climbing, hiking and touring opportunities, and is favourable for intellectual work. Accommodation and meals are provided in the school for both participants and lecturers.

Since 1958, the proceedings of the Summer School of Les Houches are edited as a series of volumes (North-Holland, publisher), which cover pedagogically all branches of physics.

Two sessions will take place in 1980, one on Defects in materials, the other on Physics of laser-plasma interaction.

The Summer School of Les Houches is an institute affiliated with the Grenoble University. The 1979 sessions receive support from the Advanced Study Institutes Programme of NATO.

APPLICATIONS

Additional information and application forms may be obtained from: *Ecole d'Eté de Physique Théorique, 74310, Les Houches, France, tel. (50) 54 4133.* Posters available upon request.

DEADLINE

APPLICATIONS AND LETTERS OF RECOMMENDATION MUST HAVE REACHED THE SCHOOL BY *March, 1979.*

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announcements

Awards

Guido Brunner, Commissioner of the European Community responsible for energy policy, has been awarded this year's Melchett Medal of the Institute of Fuel. The award is made annually "for outstanding work, whether in research, administration, construction, or other professional activity involving the scientific preparation or use of fuel, the results of which have recently been made available for the benefit of the community".

Dr David Arthur Davies, Secretary-General, World Meteorological Organization, has been awarded the Gold Medal of Merit of the Czechoslovak Academy of Sciences, Prague.

The 1977 Kalinga Prize has been awarded by a UNESCO international jury to **Fernand Seguin** in recognition of his life's work in popularising science.

Nominations are invited for the 5th Marconi International Fellowship Award. The Fellowship aims to commission creative scientific works that will add to knowledge and understanding of how communications sciences and technologies can be applied to improvement of human life. Contact Walter Orr Roberts, 1919 Fourteenth St, No. 811, Boulder, Colorado 80302.

The Council of the Royal Society are inviting nominations for the 1979 Royal Society Esso Award for the Conservation of Energy which should be sent to R. W. J. Keay, The Royal Society, 6 Carlton House Terrace, London SW1, UK, by 6 February 1979. The award is for outstanding contributions to the advance of science or engineering or technology leading to the more efficient conversion or use of any form of energy, and consists of a gold medal and a prize of £1,000.

The World Phosphate Rock Institute has created the Imphos Award to extend the impact of the First International Congress on Phosphorus Compounds and to foster research on their non-fertiliser uses. The first award will be granted in 1979. Applications to IMPHOS; 8 rue de Penthièvre, 75008 Paris, France.

The Foulkes Foundation is a registered charity which aims to further medical research. The fellowship scheme provides financial support for recently qualified science graduates with research experience who want to study medicine and for medical graduates who want to take a science degree. In both cases the graduates must intend to do medical research after qualification. For further information contact: The Secretary, Foulkes Foundation Fellowship Scheme, The Ciba Foundation, 41 Portland Place, London W1, UK, enclosing a s.a.e. The closing date for applications is 31 March 1979. The 1978 Foulkes Foundation Fellows are **G. Belcher, C. J. Derrett, Catherine T. Elder, T. S. J. Elliott, P. R. Hearn, M. J. C. Lemon, Isabella McMillen** and **D. C. Talbot**.

Meetings

18–20 December, **Palaentological Association Annual Meeting**, Keele (Dr P. D. Lane, Dept of Geology, University of Keele, Staffs, UK).

4–5 January, **Laser–Plasma Interactions**, Bangor (T. P. Hughes, Dept of Physics, University of Essex, Colchester, Essex, UK).

23–25 January, **Inter-Agency Group on Biometeorology**, Geneva (WMO, Case Postale No. 5, CH-1211, Geneva 20, Switzerland).

29 January–2 February, **RA IV Working Group on Hydrology**, Mexico City (WMO, Case Postale, No. 5, CH-1211, Geneva, Switzerland).

29 January–24 February, **Approaches to the Molecular Genetics of Oncogenic Viruses by DNA Cloning and DNA Injection into Mice Blastocysts**, Rome (Prof Enrico Calef, Centro Acidi Nucleici del CNR, Via G Romagnosi 18/A, 00196 Rome, Italy).

12 February, **Second-order Logic and the Foundations of Mathematics**, London (The British Society for the Philosophy of Science, Mr A. Young, School of Social Sciences, University of Sussex, Falmer, Brighton, UK).

15–16 February, **Immunodeficient Animals in Cancer Research**, London (The Information Officer, MRC laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey, UK).

12–23 February, **World Climate Conference**, Geneva (WMO, Case Postale No. 5, CH-1211 Geneva 20, Switzerland).

12 March, **AGM The British Society for the Philosophy of Science**, London (Mr A. Young, School of Social Sciences, University of Sussex, Falmer, Brighton, Sussex, UK).

20 March, **Mathematical Modelling of Large Scale Accidents and the Environment**, Cambridge (The Secretary, The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex, UK).

21 March, **Models of Estuaries: Flow and Pollution**, Edinburgh (The Secretary, The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex, UK).

26–30 March, **Electron Spin Resonance of Transition Metal Ions in Inorganic and Biological Systems**, Nottingham (Miss G. B. Howlett, The Chemical Society, Burlington House, Piccadilly, London W1, UK).

3–5 April, **The Chemical Society and the RIC, Annual Chemical Congress**, Bristol (Dr John Gibson, The Chemical Society, Burlington House, Piccadilly, London W1, UK).

9–11 April, **11th National Atomic and Molecular Physics Conference**, Liverpool (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

27–31 May, **International Prostaglandin Congress**, Washington, D.C. (Prof J. Martyn Bailey, PO Box 40591, Georgetown University Medical Center, Washington, D.C. 20016).

29 May–1 June, **Radioimmunoassay of Drugs and Hormones**, Gardone Riviera (Fondazione Giovanni Lorenzini, Via Monte Napoleone, 23 20121 Milan, Italy).

8–10 June, **Multidisciplinary Aspects of Brain Tumour Therapy**, Pavia (Fondazione Giovanni Lorenzini, Via Monte Napoleone, 23, 20121 Milan, Italy).

11–15 June, **14th International Microwave Power Symposium,** Monaco (Comite Francais d'Electrothermie, 79 rue de Miromesnil, 75008 Paris, France).

11–15 June, **3rd International Symposium on Purine Metabolism in Man,** Madrid (Unidad Metabolica, Fundacion Jimenez Diaz, Universidad Autonoma, Avida, Reyes Catolicos, 2, Madrid-3, Spain).

26–28 June, **Power from Sea Waves,** Edinburgh (The Secretary, The Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex, UK).

18–20 July, **Internal Friction and Ultrasonic Attenuation in Solids,** Manchester (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

15–19 July, **International Conference on Clinical Aspects of Cyclic Nucleotides,** Vail, Colorado (Howard Sands, National Jewish Hospital and Research Center, 3800 East Colfax Ave, Denver, Colorado 80206).

16–20 July, **Molecular Biology of Small DNA Tumour Viruses** (This replaces the Cold Spring Harbor DNA Tumour Virus Meeting) Cambridge (ICRF DNA Tumour Virus Meeting Secretary, ICRF, Lincoln's Inn Fields, London WC2, UK).

23–26 July, **Synthesis in Organic Chemistry, Cambridge** (Dr Malcolm Robinson, The Chemical Society, Burlington House, Piccadilly, London W1, UK).

20–23 August, **1st European Symposium on Organic Chemistry,** Cologne (Dr W. Fritsche, c/o Gesellschaft Deutscher Chemiker, PO Box 90 04 40, D-6000 Frankfurt/Main 90, FRG).

3–7 September, **Practical Applications and Design of Fibre Composites,** Cambridge (Dr Peter Beaumont, Dept of Engineering, University of Cambridge, Trumpington St, Cambridge, UK).

22–25 October, **Conference on Reclamation of Contaminated land,** Eastbourne (Miss J. Bovier, Conference Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).

10–16 February 1980, **6th International Congress of Endocrinology,** Box 611E, GPO, Melbourne 3001, Australia.

7–17 July 1980, **26th International Geological Congress,** Paris (Secretariat Général du 26ème Congrès géologique international, Maison de la Géologie, 77–79, rue Claude Bernard, 75005 Paris, France).

22–27 July 1980, **International Cyclic Nucleotide Conference,** Brussels (Dr J. E. Dumont, IRIBHN, School of Medicine, 2 rue Evers, B-1000 Brussels, Belgium).

3–9 August 1980, **16th International Congress of Entomology,** Kyoto (16th International Congress of Entomology, c/o Kyoto International Conference Hall, Takara-ike, Sakyo-ku, Kyoto 606, Japan).

17–22 August 1980, **6th International Histochemistry and Cytochemistry Congress,** Brighton (The Administrator, Royal Microscopical Society, 37/38 St Clements, Oxford, UK).

22–26 September 1980, **International Union for Vacuum Science, Technique and Applications,** Cannes (Societe Francaise du Vide, 19 rue du Renard, 75004 Paris, France).

22–26 September 1980, **3rd International Congress on the History of Oceanography,** Woods Hole (Daniel Merriman, 298 Sperry Road, Bethany, Connecticut 06525).

21–28 August 1981, **13th International Botanical Congress,** Sydney (Dr W. J. Cram, University of Sydney, New South Wales 2006, Australia).

Reports & publications

UK & Ireland—October

Pharmaceutical Prices: A Continental View. By Dr. Klaus von Grebmer. Pp. 24. (Reprinted in translation from an article in *Die Pharmazeutische Industrie.*) (London: Office of Health Economics, 162 Regent Street, W1, 1978.) £1. [2010

Natural Environment Research Council. Annual Report of the Institute of Terrestrial Ecology for 1977. Pp.viii+93. (Cambridge: Institute of Terrestrial Ecology, 1978.) £4 net [2010

National Radiological Protection Board. NRPB-R72: A Limited Survey and Evaluation of Ultraviolet Radiation Hazards in University Laboratories By A. F. McKinlay, F. Harlen and L. M. Clark Pp 16. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1978.) Obtainable from HMSO, London. £1. [2310

Information Privacy Integrity, Availability, Security, Vol 1, No. 1, September 1978. Edited by D. Sizer Pp. 1-48 Published every other month. Annual subscription: £35, $91. (Haywards Heath, Sussex. IPC Business Press (Sales and Distribution), Ltd, Oakfield House, Perrymount Road, 1978.) [2310

Tropical Products Institute. The Small-Scale Manufacture of Soap. an Economic Study. By Penelope A. Marx. (G118) Pp v+46. (London: Tropical Products Institute, 56/62 Grays Inn Road, WC1, 1978.) £1. [2310

Institute of Geological Sciences Annual Report for 1977 Pp x+226. (London Institute of Geological Sciences, 1978.) £4.50. [2410

Agricultural Development and the Rural Poor Declaration of Policy and Guidelines for Action Edited by Guy Hunter. Pp.xii+113 (London Overseas Development Institute, 10-11 Percy Street, W1, 1978.) £1.50 [2510

Thames Water—Annual Report and Accounts for the year ended 31st March, 1978. Pp. 76 (London. Thames Water Authority. New River Head, Rosebery Avenue, EC1, 1978.) [2510

Council for National Academic Awards. Directory of Postgraduate and Post-Experience Courses, 1978-79. Pp. 88. Directory of First Degree Courses, 1978-79. Pp. 184 (London Council for National Academic Awards, 344-354 Gray's Inn Road, WC1, 1978.) [2510

The Polytechnic of North London. Prospectus 1979-80. Pp 114. (London. The Polytechnic of North London, Holloway, N7, 1978.) [2510

Identification of Larval Ticks Found on Small Mammals in Britain. By K. R. Snow. Pp. 14. (Bury St. Edmunds, Suffolk. Publications Officers, Mammal Society, Larkmead, Barton Mills, 1978.) 25p+10p. p+p [2710

Natural Environment Research Council. Chemistry in the Institute of Terrestrial Ecology By S. E. Allen. Pp. 31 (Cambridge: Institute of Terrestrial Ecology, 68 Hills Road, 1978.) 75p net. [3010

National Coal Board Mining Research and Development Establishment Projects 1977/78. Pp. 49. Annual Report, 1977/78. Pp. 28. (Burton-on-Trent, Staffs.: Mining Research and Development Establishment, Ashley Road, Stanhope Bretby, 1978.) [3110

Other countries—October

Application of the International Classification of Diseases to Dentistry and Stomatology, ICD-DA Second edition. Pp. 144 (Geneva: WHO, London: HMSO, 1978) Sw.fr.20, $11. [2010

Smithsonian Contributions to Zoology, No 278 A Catalogue of the Type-Specimens of Recent Cephalopoda in the National Museum of Natural History. By Clyde E Roper and Michael J. Sweeney. Pp.iii+19 (Washington, DC: Smithsonian Institution Press, 1978. For sale by US Government Printing Office) [2310

The European Community Today and Tomorrow. Pp. 64 (Luxembourg. Office for Official Publications of the European Communities, Boite Postale 1003; 1978.) Bfr 32; 60p. [2310

United States Department of the Interior: Geological Survey. Professional Paper 935-C Crystallization and Differentiation of the Alae Magma, Alae Lava Lake, Hawaii. By Thomas L. Wright and Dallas L Peck Pp iv+20. Professional Paper 1053 Potential Effects of Deep-Well Waste Disposal in Western New York. By Roger M Waller, John T. Turk and Robert J. Dingman. Pp.v+39. Professional Paper 1055. Reconnaissance Geology and Geochronology of the Precambrian of the Granite Mountains, Wyoming By Zell E. Peterman and R. A. Hildreth. Pp.iii+22 (Washington, DC US Government Printing Office, 1978.) [2310

United States Department of the Interior: Geological Survey Bulletin 1444. Geology and Thermal History of Mammoth Hot Springs, Yellowstone National Park, Wyoming By Keith E Bargar. Pp.vii+55. (Washington, DC.: US Government Printing Office, 1978.) [2410

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Oregon Regional Primate Research Center. Progress Report for 1977. Pp 40. (Beaverton, Oregon: Oregon Regional Primate Research Center, 505 N W. 185th Avenue, 1978) [2410

Mutants on Physiological Research of Crop Plants Report of Symposium held on July 22-23, 1976. (Gamma Field Symposia, No. 15) Pp.iv+89. (Ohmiya-machi, Ibaraki-ken, Japan Institute of Radiation Breeding, NIAS MAF, 1978) [2510

United States Department of the Interior: Geological Survey Water-Supply Paper 2048. Water Quality in the Ozark National Scenic Waterways, Missouri. By James H Barks. Pp.vii+57 Professional Paper 1022-C. Postglacial Volcanic Deposits at Mount Baker, Washington, and Potential Hazards from Future Eruptions. By Jack H. Hyde and Dwight R. Crandell Pp.v+17+1 plate. Professional Paper 1051. Field Determination of Vertical Permeability to Air in the Unsaturated Zone. By Edwin P. Weeks Pp.iv+41. Professional Paper 1068: Effects of Permafrost on Stream Channel Behaviour in Arctic Alaska By Kevin M. Scott. Pp.iii+19. (Washington, DC.: US Government Printing Office, 1978.) [2510

Deutsches Hydrographisches Institut, Hamburg. Jahresbericht 1976/77. Pp. 137.) Hamburg: Deutsches Hydrographisches Institut, 1978.) [2510

World Health Organization Technical Report Series, No 623: Induced Abortion: Report of a WHO Scientific Group. Pp. 65. Sw.fr.7. Public Health Papers, No. 69: Poverty, Development and Health Policy. By Brian Abel-Smith and Alcira Leiserson. Pp. 109. Sw.fr 10; $5. Geneva. WHO, London. HMSO, 1978) [3010

CERN—European Organization for Nuclear Research. CERN 78-07: The Nodal System for the SPS. By M C Crowley-Milling and G. C. Shering. Pp.vii+98. (Geneva. CERN, 1978.) [3010

The Worldwide Loss of Cropland. By Lester R. Brown. Pp. 48. (Worldwatch Paper No. 24.) (Washington, DC.: Worldwatch Institute, 1776 Massachusetts Avenue, N.W, 1978) $2. [3110

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Recent scientific and technical books

Applied biology

ANDERSEN, K. Lange, MASIRONI, R., RUTENFRANZ, and SELIGER, C. In collaboration with DEGRE, S., TRYGG, K., and ORGIM, M. Habitual Physical Activity and Health. (WHO Regional Publications. European Series No. 6.) Pp.188. ISBN-92-9020-106.1. (Copenhagen. WHO, Regional Office for Europe, London. HMSO, 1978.) Sw.fr.25, $12.50.

BARLATI, Sergio, and GIULI-MORGHEN, Carlo (edited by). Avian RNA Tumor Viruses. (Proceedings of the ICREW-EMBO Workshop, Pavia, 9th-12th September 1977.) Pp.xv+355. (Padua: Piccin Medical Books, 1978.) $30.

BRANDLY, C. A., and CORNELIUS, Charles E. (edited by). Advances in Veterinary Science and Comparative Medicine, Vol. 22. Pp.xii+325. ISBN-0-12-039222-4. (New York and London. Academic Press; a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) $35, £22.75.

BROOKS, Geo. F., GOTSCHLICH, Emil C., HOLMES, King K., SAWYER, William D., and YOUNG, Frank E. (edited by). Immunobiology of *Neisseria gonorrhoeae*. (Proceedings of a Conference held in San Francisco, California, 18-20 January 1978). Pp.xxii+399. ISBN-0-914826-18-2. (Washington, DC.: American Society for Microbiology, 1978.) $15.

CALMAN, Kenneth C., and PAUL, John. An Introduction to Cancer Medicine. Pp.200. ISBN-0-333-21277-0. (London and Basingstoke: The Macmillan Press, Ltd., 1978.) Hard cover £8; Paperback £3.95.

COCHRAN, Alistair J. Man, Cancer and Immunity. Pp.xi+206. ISBN-0-12-177550-X. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) £7.80; $16.15.

CORNER, M. A., BAKER, R. E., van de POLL, N. E., Swaab, D. F., and UYLINGS, H. B. M. (edited by). Maturation of the Nervous System. (Progress in Brain Research, Vol. 48.) Pp xii+425. ISBN-0-444-80036-0. (Amsterdam, New York and Oxford: Elsevier Scientific Publishing Company, 1978.) Dfl. 160; $71.25.

COTTRAL, George E. (edited by). Manual of Standardized Methods for Veterinary Microbiology. Pp.731. ISBN-0-8014-1119-X. (Ithaca and London: Comstock Publishing Associates, a Division of Cornell University Press, 1978.) $35.

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PORTNEY, Paul R. (edited by). Current Issues in U.S. Environmental Policy. Pp.xv+207. ISBN-8018-2119-3. (Baltimore, Md. and London: The Johns Hopkins University Press, 1978. Published for Resources for the Future.) Cloth £10.50; Paper £3.50.

PRESTON, David A. Farmers and Towns: Rural-Urban Relations in Highland Bolivia. Pp.xi+196. ISBN-0-86094-008-X. (Norwich: Geo Abstracts, Ltd., University of East Anglia, 1978.) Paperback £4.95, $9.90. Hardback £6.50; $13.

RAPP, Jr., George, and ASCHENBRENNER, S. E. (edited by). Excavations at Nichoria in Southwest Greece. Vol. 1: Site, Environs, and Techniques. Pp.xxviii+339+plates. ISBN-0-8166-0824-5. (Minneapolis. The University of Minnesota Press, 1978.) $29.75.

RIEFENSTAHL, Leni. Coral Gardens. Translated from the German by Elizabeth Walter. Pp.223. ISBN-0-00-216129-X. (London Collins, 1978.) £15 net.

ROSEN, R. D. Psychobabble. Fast Talk and Quick Cure in the Era of Feeling. Pp.xi+233. ISBN-0-7045-0221-6. (London. Wildwood House, New York: Atheneum, 1978.) £5.95.

RUSSELL, Peter. The TM Technique. An Introduction to Transcendental Meditation and the Teachings of Maharishi Mahesh Yogi. Pp.205. ISBN-0-7100-0068-5. (London, Henley and Boston, Mass.. Routledge and Kegan Paul, 1978.) £1.95.

SLESSER, Malcolm. Energy in the Economy. Pp.x+164. ISBN-0-333-21495-1. (London and Basingstoke. The Macmillan Press, Ltd., 1978.) Hardcover £8.95; Paperback £3.95.

SMALLMAN, Clare, and BELLAMY, David. Botanic Action with David Bellamy. Pp.64. ISBN-0-09-134141-8. (London: Hutchinson of London, in association with National Extension College and Thames Television.) Cased £3.95, Paper £1.95.

STARK, Barbara L., and VOORHIES, Barbara (edited by). Prehistoric Coastal Adaptations The Economy and Ecology of Maritime Middle America. (Studies in Archaeology.) Pp.xx+313. ISBN-0-12-663250-2. (New York and London. Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) $21, £13.65.

STEINER, George. On Difficulty and Other Essays. Pp.xi+209. ISBN-0-19-212208-8 (Oxford and Melbourne: Oxford University Press, 1978.) £5.50 net.

STERLING, Keir B. Last of the Naturalists. The Career of C. Hart Merriam. (Natural Sciences in America) Pp.xii+472 ISBN-0-405-10307-7. (New York: Arno Press, a New York Times Company, 1977.) $23.

THOMSON, Garry. The Museum Environment. (Conservation in the Arts, Archaeology and Architecture.) Pp.xiv+270. ISBN-0-408-70792-5. (London and Boston Mass.. Butterworths, 1978. Published in association with The International Institute for Conservation of Historic and Artistic Works.) £16; $32.

WALLACE, David Rains. The Dark Range. a Naturalist's Night Notebook. Pp.xi+131. ISBN-0-87156-251-0 (San Francisco: Yolla Bolly Press Book, Sierra Club Books, 1978.) Cloth $15. Paper $8.95.

WERSKEY, Gary. The Visible College. Pp.376. ISBN-0-7139-08262. (London. Allen Lane, Penguin Books, Ltd, 1978) £10 net.

WIENER, A. Magnificent Myth Patterns of Control in Post-Industrial Society. (Pergamon International Library of Science, Technology, Engineering and Social Studies) Pp.xviii+413. ISBN-0-08023100-4. (Oxford and New York Pergamon Press, 1978.) Hard £15; Flexi £5.

WINNER, Langdon. Autonomous Technology Technics-out-of-Control as a Theme in Political Thought Pp.x+386. ISBN-0-262-73049-9. (Cambridge, Mass. and London. The MIT Press, 1978) np

WOLF, Josef. The Dawn of Man. Edited and adapted by Peter Andrews, Robin Harvey, Colin Ridler and Christopher Stringer Pp.231. ISBN-0-500-01-198-2. (London Thames and Hudson, Ltd. 1978.) £3.95 net.

WOOD, Denis (compiled by) Poets in the Garden. An Anthology of Garden Verse. Pp.126. ISBN-0-7195-3562-X. (London John Murray (Publishers), Ltd., 1978.) £2.95 net.

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UNIVERSITY OF EDINBURGH

TECHNICIAN

Required for the DEPARTMENT OF CHEMISTRY in the S R.C supported Edinburgh University Fourier Transform NMR Service. The successful applicant will be required to assist in the operation of the Varian XL—100 and Bruker WH 360 multinuclear magnetic resonance spectrometers and in the general running of the service under the direction of Dr I H. Sadler. Experience in the field of high resolution NMR and with Fourier Transform NMR spectrometers would be an advantage Qualifications H N C or equivalent. The appointment will be for four years with the possibility of renewal Salary on scale £3,654 to £4,365 per annum (under review). Applications together with the names of two technical referees should be submitted as soon as possible Ref NT060

Application forms for the above post can be obtained from the Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS Telephone 031-556 2930 8638(A)

UNIVERSITY OF CAMBRIDGE

INSTITUTE OF ASTRONOMY

RESEARCH APPOINTMENTS IN THEORETICAL ASTRONOMY

Applications are invited for S R C.-supported postdoctoral positions for research in theoretical astronomy Appointments will be for two years in the first instance, with possible extension for a third year The salary scale (under review) is £4,113 (age 25) to £6,555 (age 35), plus U S S benefits The preferred starting date is on or before October 1979 Applications should be sent to Professor M J Rees, Institute of Astronomy, Madingley Road, Cambridge CB3 0HA by January 30, 1979 Applicants should themselves please ask three referees to write to Professor Rees by the same date

DATA ANALYSIS FOR X-RAY ASTRONOMY

An S R C -supported post is available from October 1979 for a postdoctoral worker to assist in the analysis of X-ray astronomical data The work arises out of collaboration with groups elsewhere Travel to the groups involved—and, in particular, several visits to the U S of about one month duration—will be necessary The post is tenable for up to three years, at a starting salary (under review) of up to £4,631 (age-point 27) plus U S S benefits Further information can be obtained from Dr A C Fabian, Institute of Astronomy, Madingley Road, Cambridge CB3 0HA, and applications must be received by January 30, 1979 Applicants should themselves ask three referees to write to Dr Fabian by the same date.

8644(A)

UNIVERSITY OF EDINBURGH

DEPARTMENT OF MOLECULAR BIOLOGY

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the post of Postdoctoral Research Fellow to work on a project funded by the S R C. The project is concerned with the separate isolation of copy DNA sequences from *Xenopus* oocyte messenger RNA by molecular cloning and applicants should have experience of nucleic acid research and/or molecular cloning techniques

The salary is on Range IA (£3,883 to £6,555 per annum) and the post is for three years

Applications, including a curriculum vitae and the names and addresses of two referees, should be sent to Dr P J Ford, Department of Molecular Biology, King's Buildings, Mayfield Road, Edinburgh EH9 3JR Please quote Reference 5035 8610(A)

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appointment to the following

FACULTY OF SCIENCE

A.R.G.C. RESEARCH FELLOW— BIOLOGICAL CHEMISTRY

A Research Fellow is required to work in the area of endogenous growth control substances in Eucalyptus species under the direction of Associate Professor W D Crow (Department of Chemistry) and Dr D M Paton (Department of Botany) Candidates should hold a Ph D. degree or equivalent and have some experience in working with plant material in a chemically-oriented manner Knowledge of, or experience in plant physiology/biochemistry would be of considerable value The Fellow will work in collaboration with senior members of the Departments of Botany, Chemistry and Forestry, full research facilities are available, including access to high-field n m r

The position will be funded by the Australian Research Grants Committee for three years from February 1, 1979

CLOSING DATE **DECEMBER 9, 1978.**

Salary on appointment will be in accordance with qualifications and experience within the range $A15,179 to $A17,220 per annum. Current exchange rates $A1 $US1 14 £0.58

Reasonable appointment expenses are paid Superannuation benefits are available for applicants who are eligible to contribute Assistance with housing is provided for an appointee from outside Canberra

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should obtain further particulars from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 8646(A)

BIOCHEMISTRY

UNIVERSITY OF CALIFORNIA, SANTA CRUZ

Applications are invited for new faculty position jointly with Merrill College in Chemistry at University of California, Santa Cruz Preference will be given to candidates with research interests in area of biochemical approaches to cellular regulation. Teaching duties include both undergraduate and graduate courses in metabolism and regulation. Likely level of this appointment is Assistant Professor

Send resume, brief outline of future research/teaching plans, and three letters of reference to

Thomas Schleich, Chairperson
Biochemistry Search Committee
Division of Natural Sciences
University of California
Santa Cruz, California 95064

Closing date for this position is January 17, 1979.

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8665(A)

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Experimental High Energy Physicist

The National Research Council of Canada's Division of Physics is located in Ottawa, Canada. The High Energy Physics Laboratory requires an experienced and dynamic experimental high energy Physicist with proven leadership ability to work with a team of eight experimentalists, three theorists and several Research Associates. The group includes staff of Carleton University (also in Ottawa) which has a sophisticated high energy instrumentation capability.

Studies are currently being carried out in meson spectroscopy at high energy accelerators, and meson physics at intermediate energy facilities. The successful applicant will be responsible for initiating and carrying out advanced research at modern accelerators such as SLAC and FERMILAB

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NRC

Apply in writing giving complete details of education and experience to the Employment Officer, National Research Council of Canada, Ottawa, Ontario, Canada, K1A OR6 In reply please quote P-77-1057

Please reply by January 9, 1979.

8597(A)

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The candidate should be able to conduct independent research programmes.

Past or present experience in pharmaceutical industry is essential.

An excellent salary and comprehensive benefit programme accompany this position.

Please send curriculum vitae to P.O. Box No. 8633, 3 Dyers Buildings, Holborn EC1N 2NR.

8633(A)

THE BRITISH INDUSTRIAL BIOLOGICAL RESEARCH ASSOCIATION

Toxicologists

The Organisation: A research association involved in the toxicological assessment of environmental chemicals with associated basic/fundamental research programmes. The Association is supported by Industry and Government and enjoys an international reputation.

The Department: Responsibility for all short- and long-term safety evaluation studies.

The Positions: Involving supervision and management of short-and long-term toxicity and carcinogenicity programmes and multigeneration studies.

Qualifications: A first or second degree in a biological subject is required and preference will be given to applicants with revelant experience.

Employment Benefits: The salary will be in the range £4,134 to £6,180, dependent upon age, qualifications and experience. A contributory pension and assurance scheme is operated.

Applications: With curriculum vitae to—

The Secretary
The British Industrial Biological Research Association
Woodmansterne Road
Carshalton, Surrey SM5 4DS

8617(A)

Animal Health Development

May & Baker Ltd., is an international Company manufacturing and marketing a wide range of research based chemicals, including animal health products

A vacancy has arisen, based at the Company's Research Station at Ongar, Essex, for a technical officer to join a team working on development of new animal health products. The work will include planning of product development, participating in field trials and preparation of technical literature. It will also involve liaison with other units within the Company and overseas. Frequent travelling in the U.K. and some overseas travelling will be necessary.

Applications are invited from science graduates with relevant experience, preferably in an industrial environment. A current driving licence is essential.

Benefits of employment include flexible working hours, bonus scheme and contributory pension scheme with life assurance. Assistance with relocation will be given where appropriate.

Please telephone 01-592 3060 Ext. 2506 for an application form or write, with full details of age, experience and present salary to Mrs. P. M Clark, Personnel Officer,

M&B May&Baker

May & Baker Ltd., Dagenham, Essex, RM10 7XS quoting reference No. 179/N/1.

8649(A)

HOUGHTON POULTRY RESEARCH STATION
GENETICIST

A vacancy exists in the Leukosis Experimental Unit for a geneticist to study the inheritance and nature of resistance of chickens to oncogenic and other viruses, and to assist in the formulation of breeding programmes for the Institute's flocks

Applicants must have a first or upper second class honours degree, with genetics as a major subject in the first or a higher degree, and preferably a Ph D or at least 3 years' relevant postgraduate experience A minimum of 4 years' relevant postgraduate experience is required for the S S O grade

The appointment will be to the Higher Scientific Officer grade (£4,101 by 8 to £5,448) or the Senior Scientific Officer grade (£5,154 by 8 to £6,898), grade and starting salary depending upon experience Non-contributory superannuation scheme.

Further particulars and application forms from the Station Secretary, Houghton Poultry Research Station, Houghton, Huntingdon, Cambs. PE17 2DA Closing date for applications is January 10, 1979.

8507(A)

UNIVERSITY OF GLASGOW
DEPARTMENT OF BIOCHEMISTRY
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a Post-doctoral Research Assistant supported by the M R.C. to work with Dr H. G. Nimmo on the purification and regu-latory properties of the mitochondria glycerol phosphate acyltransferase from ammalian liver Prior experience of working with membrane-bound enzymes would be an advantage.

The appointment will initially be for one year, from January 1, 1979 renewable for two further years subject to satisfactory progress

Salary within range £3,883 to £4,88 on Range 1A of the salary scale for Research and Analogous staff according to age and experience Superannuation Scheme will apply.

Applications, with a curriculum vitae and the names of two referees should be sent to Dr H G. Nimmo Department of Biochemistry, University of Glasgow, Glasgow G1 8QQ, by December 22, 1978.

In reply please quote Ref No 4319M.

8641(A)

HERIOT-WATT UNIVERSITY

DEPARTMENT OF BREWING & BIOLOGICAL SCIENCES

RESEARCH ASSOCIATE—ESTUARINE ALGAE

Applications are invited for the post of N.E.R.C. Research Associate to work on the ecology of intertidal algae in a small estuary polluted by very low pH effluents with very high nutrient status and elevated levels of certain metals. The project is under the supervision of Dr M. Wilkinson. Applicants should have an honours degree in a biological subject. The successful candidate may be eligible to register for a higher degree.

Initial salary will be in the range £3,384 to £3,883 per annum and the post is for three years commencing January 1, 1979.

Informal enquiries may be made to Dr M. Wilkinson, Department of Brewing & Biological Sciences, Tel 031-225 8432, ext. 347.

Application forms are available from the Staff Officer, Chambers Street, Edinburgh EH1 1HX to whom completed forms should be returned by Friday, December 15, 1978. Ref. 14/78 8593(A)

UNIVERSITY OF VICTORIA, B.C. CANADA

DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY

Applications are invited for the position of

ASSISTANT PROFESSOR

beginning July 1, 1979. Candidates should have strong teaching and research interests in one or more of the following fields: immunology, protein biochemistry, molecular genetics (recombinant DNA), virology. The successful applicant will be required to teach at the undergraduate and graduate levels and carry on an active research program. A Ph.D. is required and postdoctoral experience desirable.

Applications, including a *curriculum vitae*, list of publications, a brief statement of research plans, and the names of three referees who have been requested to send letters of recommendation should be sent to Professor A. T. Matheson, Chairman, Department of Biochemistry and Microbiology, University of Victoria, P.O. Box 1700, Victoria, B.C., Canada V8W 2Y2, as soon as possible 8663(A)

UNIVERSITY OF GENEVA (SWITZERLAND)

POSTDOCTORAL RESEARCH APPOINTMENT

is immediately available at the Laboratory for Applied Chemistry for an *inorganic chemist*, *solid state chemist* or *solid state physicist* interested in joining an experimental programme (Swiss National Science Foundation) on the synthesis and the properties of ferroelectric and magnetically ordered ferroelectric, magnétoelectric materials. Renewal of the appointment is possible from year to year.

Applicants should submit résumé, publication list and names of two referees to:

Professor H. Schmid,
Applied Chemistry Laboratory,
University of Geneva,
30, quai Ernest-Ansermet,
CH-1211 Geneva 4. 8645(A)

THE QUEEN'S UNIVERSITY OF BELFAST

RESEARCH OFFICER

DEPARTMENT OF MEDICAL GENETICS

Applicants must hold a University degree, or equivalent qualification, and have experience in Biochemistry, or Chemistry. The Department is involved in culture of various tissue including blood, skin, and aminotic fluid cells. The Research Officer will be responsible for initiating biochemical techniques applicable to tissue culture. Knowledge and experience in enzyme electrophoresis, column chromatography, would be an advantage. Duties will include participation in the Department's research and teaching programmes and assisting with a number of postgraduate research projects.

Salary scale (under review) £3,885 to £6,555 per annum with superannuation.

Further particulars and application forms may be obtained from the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. Closing date: December 22, 1978. (Please quote Ref 78/N). 8624(A)

UNIVERSITY OF BRISTOL

DEPARTMENT OF PATHOLOGY

POSTDOCTORAL RESEARCH ASSISTANT

(£4,382 to £5,367)

Applications are invited for the above post from biological scientists with experience in cell membrane or protein biochemistry, and immunology. The successful applicant will join a new M.R.C. funded five-year programme to investigate the use of membrane antigens as a possible EB-virus vaccine as part of a wider study, technical help will be available. The appointment will be for one year initially, renewable annually.

Replies in writing, giving the names and addresses of two referees to Professor M. A. Epstein, Department of Pathology, The Medical School, University Walk, Bristol BS8 1TD, by December 15, 1978 8614(A)

UNIVERSITY OF NEWCASTLE UPON TYNE

The Medical School

RESEARCH OFFICER

Applications are invited from suitably qualified graduates (preferably post doctoral) for a post as Research Officer in the Department of Psychiatry. The successful candidate will join the research unit of the Department and, although the work will be primarily concerned with pharmacological and biochemical research into brain neurotransmitter mechanisms, he/she will also have freedom to develop his/her own research interests.

Commencing salary according to age qualifications and experience on either the Range 1B salary scale £3,384 to £4,882 per annum or the Range 1A salary scale £3,883 to £6,555 per annum. The post is available for up to three years. Membership of the appropriate University superannuation scheme will be required.

Further enquiries may be made to Professor D. Eccleston (telephone 0632 25131, Ext. 3167). Applications (three copies) together with the names and addresses of three referees should be sent to the Senior Administrative Assistant, The Medical School, University of Newcastle upon Tyne NE1 7RU, from whom further particulars may also be obtained, not later than December 22, 1978. 8615(A)

THE UNIVERSITY OF JUBA—SUDAN

Applications are invited for the following posts in the COLLEGE OF NATURAL RESOURCES AND ENVIRONMENTAL STUDIES (Staff are urgently required in Juba by mid-February 1979).

1. PROFESSOR/ASSOCIATE PROFESSOR/LECTURER IN ANIMAL PRODUCTION.
2. PROFESSOR/ASSOCIATE PROFESSOR/LECTURER IN FISHERIES
3. PROFESSOR/ASSOCIATE PROFESSOR/LECTURER IN WILDLIFE
4. PROFESSOR/ASSOCIATE PROFESSOR/LECTURER IN FORESTRY.
5. PROFESSOR/ASSOCIATE PROFESSOR/LECTURER IN LAND USE
6. PROFESSOR/ASSOCIATE PROFESSOR/LECTURER IN ECOLOGY.
7. PROFESSOR/ASSOCIATE PROFESSOR/LECTURER IN BIOCHEMISTRY.
8. PROFESSOR/ASSOCIATE PROFESSOR/LECTURER IN PLANT PROTECTION.
9. PROFESSOR/ASSOCIATE PROFESSOR/LECTURER IN PHYSICS.
10. PROFESSOR/ASSOCIATE PROFESSOR/LECTURER IN SOIL SCIENCE.
11. PROFESSOR/ASSOCIATE PROFESSOR/LECTURER IN ANIMAL PHYSIOLOGY.

Salary scales: Professor £S5,460 per annum; Associate Professor £S4,030 to £S4,550 per annum; Lecturer £S ,500 to £S3,500 per annum (these scale exclude cost of living allowance) (£S1 equals £1 27 sterling). The British Government may supplement salaries in range £3,408 to £3,966 per annum (sterling) for married appointees or £1,092 to £1,734 per annum (sterling) for single appointees (normally free of all tax and presently under review) and provide children's education allowances and holiday visit passages. Family passages various allowances, superannuation scheme; annual overseas leave. Detailed applications (two copies), including a curriculum vitae and naming three referees, should be sent by air mail, not later than December 28, 1978 to the Secretary General, University of Juba, P.O. Box 82, Juba, Sudan. Applicants resident in U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. 8589(A)

SCIENCE, ENGINEERING AND TECHNOLOGY

The British Council

OVERSEAS CAREER SERVICE

The British Council, which has a responsibility for Britain's cultural and educational relations overseas, will have some vacancies in 1979. The Council is represented in over 80 countries. Overseas Career Service staff must expect to serve wherever they are posted, and to spend two thirds or more of their working lives abroad.

Staff joining the Overseas Career Service bring with them a wide variety of skills and experience and most appointments are made with a view to eventual promotion on merit to senior managerial posts. The Council has a continuing need for a proportion of new staff with qualifications and experience in science and engineering, as well as in the other specialist subjects of interest to the Council. English language teaching and librarianship for example.

We should like to hear from you if you have a background in science or engineering and some postgraduate experience in research, industry, education or administration. An essential requirement is the personal and managerial qualities which overseas representation demands.

Recently, most successful candidates for the Overseas Career Service have been between 25 and 32 years of age, but we should be glad to hear from younger or older candidates who have the necessary background. Only in exceptional cases is appointment offered to candidates over the age of 40.

The initial salary is in the region of £5,300 possibly higher for particularly well-qualified candidates, and terms and conditions of service are in line with those of comparable organisations in the public sector: free accommodation and overseas allowances, including children's education allowances, are provided while overseas.

For further details and an application form, please write or telephone quoting C1 to Staff Recruitment Department, The British Council, 65 Davies Street, London W1Y 2AA, telephone 01-499 8011 extension 3041

8585(A)

CSIRO AUSTRALIA

Postdoctoral Research fellow

Division of Fisheries and Oceanography Cronulla, NSW

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7000 employees—2400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: **FISHERIES BIOLOGY**

Duties: Initially to develop and supervise a research programme on the highly migratory fish species that occur in the seas adjacent to Australia. Field investigations on the Division's vessels and on fishing vessels in the open sea will be involved. Temporary posting to other parts of Australia may be required.

Qualifications: A Ph.D or equivalent in one of the ecological sciences with heavy emphasis on mathematics and statistics and the use of computers Specific training and/or experience in population dynamics is desirable.

Salary: Research Scientist: $A14829 to $A18177 p.a. or Senior Research Scientist. $A18819 to $A21543 p.a. A higher salary might be offered to a more experienced applicant

Tenure: A fixed-term appointment of 3 to 5 years, with superannuation benefits.

Applications **in duplicate**, stating **full** personal and professional details, the names and addresses of at least two professional referees, and **quoting reference number 320/809** should reach:

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, London WC2R 3EH by **29th December 1978.**

Applications in U.S.A. and Canada should be sent to:

The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S.A.

8618(A)

Camden & Islington Area Health Authority (T)

Whittington Hospital

Probationary Grade/ Basic Grade Biochemist

required for the Endocrine Laboratory in the newly built and well-equipped Pathology Department of the above University Hospital Applicants should have an appropriate Science degree. Experience, though desirable, is not essential as training will be given.

Salary on the scale £3,486 to £3,916 or £4,038 to £4,899, plus £364 London Weighting Allowance

Application forms obtainable from: Hospital Personnel Dept., Whittington Hospital, Highgate Hill, London N 19
Tel. 01-272 9679.

8655(A)

UNIVERSITY OF RHODESIA

CHAIR OF MATHEMATICS

Applications are invited for the post of Professor and Head of the Department of Mathematics which will become vacant on the retirement of the Foundation Professor, Professor R A Manwell, on December 2, 1978. The Chair may be filled from any branch of Mathematics but, other things being equal, preference will be given to a candidate who has an interest in applicable Mathematics

Qualifications Ideally, applicants should hold a doctorate and have held a senior university appointment in Mathematics.

Salary Scale (Approx. Stg equiv) £10,291 by £387 to £12,226.

Conditions. Appointment will be on permanent, pensionable terms and these include family passages and allowance towards transport of effects, installation loan of up to half of one year's salary if required; unfurnished University accommodation guaranteed for a period of at least three years for persons recruited from outside Rhodesia, sabbatical leave and biennial visits with travel allowances, superannuation and medical aid schemes

Applications (6 copies) giving full details of personal particulars, qualifications, experience, research, publications together with the names and addresses of three referees should be sent to the Assistant Registrar (Science), University of Rhodesia, P O Box MP 167, Mount Pleasant, Salisbury, Rhodesia Overseas applicants should send an additional copy of their applications to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Further particulars may be obtained from either of the above

Closing date for receipt of applications **December** 31, 1978.

British subjects considering applying for posts in Rhodesia are urged to consult the Foreign and Commonwealth Office (telephone 01-233 8727) or their nearest British Consular Office

8630(A)

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NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

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Application forms are obtaina
from the Secretary, NIRD, Shinfie
Reading RG2 9AT. Quote referen
78/36. 8627(A

GRADUATE RESEARCH ASSI
ANT, with a degree in a mathem
ically based subject, required to wo
for two years in the Department
Thoracic Medicine with a physic
and physiologist on pulmonary
organisation in lung disease. Practi
experience in laboratory work
computing an advantage Apply
writing, with full curriculum vitae,
the Secretary, Guy's Hospital Medi
School, London Bridge SE1 9R
quoting Ref T M.1 8631(A

FACULTY POSITION IN CELL BIOLOGY

Assistant Professorship open for
cell biologist with specific training
protein biochemistry and ultrastru
tural approaches to the analysis
cell structure and function: for
ample, microtubules, microfilamen
or membrane structure. Candida
must have a Ph D. or M D, a mi
mum of two years postdoctoral tra
ing, and demonstrated productivity
original research The successful ca
didate will be expected to establish
strong independent research p
gramme and train graduate studen
Undergraduate-graduate student teac
ing responsibilities include advanc
cell biology.

Applicants should send curricul
vitae, bibliography, a brief descripti
of future research and teaching
terests, career objectives and names
three referees by January 15, 1979,
Dr Susan A. Gerbi, Cell Biolog
Search Committee, Division of Biolo
and Medicine, Brown University, P
vidence, Rhode Island 02912

Brown University is an Affirmat
Action/Equal Opportunity Employ
8625(A)

Tropical Products Institute
London

Biochemist

■ Participate in work programme relating to fish ■ Investigate methods of preservation for tropical fish and for the utilization of by-products ■ Examine ways of reducing wastage by developing novel products ■ Involvement in the training of overseas personnel possible.

☐ Degree/HNC/HND or equivalent in appropriate discipline ☐ Ability to work independently in areas of practical technical development in the UK and overseas essential ☐ Age normally under 30 ☐ Appointment as Higher Scientific Officer (£4560–£5910) or Scientific Officer (£3300–£4880) ☐ Ref: SA/93/JD.

■ *For application form see below*

■ Application forms (for return by 22 December 1978) from Miss C A Hall, Tropical Products Institute, 127 Clerkenwell Road, London, EC1R 5DB.

8616(A)

Science Group
CIVIL SERVICE

UNIVERSITY OF SYDNEY LECTURESHIPS (TWO) IN PHYSICS

Appointments will be made in the eld of solar energy physics.

Salary range: $A15,179 to $A19,940 er annum.

The positions may be filled either a tenured basis or, in the first stance, for a probationary period of ree years with the possibility of nure being given during or shortly ter that period, or in certain cases turn fares.

Applications, including curriculum tae, list of publications and names three referees, by **January 31, 1979** the Registrar, University of Sydney, .S.W. 2006, Australia, from whom rther information is available. Inrmation also available from the ssociation of Commonwealth Univeries (Appts), 36 Gordon Square, ondon WC1H 0PF. Details of teachg and research duties, etc. may be tained from the Head of the School Physics, in the University.

8652(A)

ROTHAMSTED EXPERIMENTAL STATION
HARPENDEN, HERTS AL5 2JQ

Applications are invited from suitably qualified scientists for the post of Head of the Soil Microbiology Department to succeed Dr P. S. Nutman, F.R.S., who retires in Autumn 1979.

The Soil Microbiology Department has over 20 scientific staff and receives many visitors. The scientist appointed will be expected to lead actively the study of soil micro-organisms of agricultural significance and to develop this research in collaboration with other relevant disciplines at Rothamsted.

Appointment in the grade of S.P.S.O., £10,043 by two increments to a maximum of £11,300. Non-contributory superannuation.

Apply in writing to the Secretary giving names and addresses of two referees and quoting Ref. No. 378 by January 12, 1979. Further details on request.

8635(A)

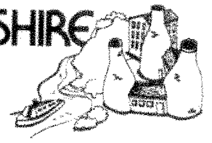
There is a vacancy for a BIOLOGIST in *Nature's* London office. He or she would join the team which assesses the suitability of biological manuscripts for publication; there would also be opportunities to work with members of the *Nature* staff in compiling other sections of the journal. The job might appeal to the holder either of a bachelor's or an advanced degree.

Write to:
The Editor
NATURE
MACMILLAN JOURNALS LIMITED
4 Little Essex Street
London WC2R 3LF

enclosing a curriculum vitae and any other material you consider relevant.

8577(A)

NORTH STAFFORDSHIRE HEALTH DISTRICT



TOP GRADE BIOCHEMIST

Applications are invited from science graduates for the post of Top Grade Biochemist at the North Staffordshire Hospital Centre. Wide experience is essential and the possession of higher qualifications is desirable.

The successful candidate will participate in the routine running of the department and will be expected to pursue his or her own research interests. There are close relations with the University of Keele and opportunities exist for participation in Post Graduate teaching and lecturing. The department, which is one of the largest in the country, is well equipped and extensively automated and computerised. It is situated in a modern building.

Those interested in visiting the laboratory should contact Dr. J. A. H. Brown, Consultant Pathologist, Telephone 0782-49144 Ext. 4669.

Further details regarding the appointment are available from the District Personnel Officer, The Limes, Hartshill Road, Hartshill, Stoke-on-Trent, ST4 7PS.

Closing date for completed applications: January 31, 1979.

8594(A)

Staffordshire Area Health Authority

CSIRO
AUSTRALIA
Postdoctoral Research fellow
Division of Mineralogy North Ride, NSW

CSIRO has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: **ISOTOPE GEOCHEMISTRY**

General: The Division's research programme includes development of surface and subsurface geochemical techniques for mineral exploration. Isotopic methods are being tested, and significant progress has been made in applying lead isotopes to exploration for stratiform base metal sulfide deposits.

Duties: To conduct lead isotope measurements on samples from a variety of geological environments and ore types, and to evaluate the applicability of results in mineral exploration. Field work and close liaison with mining companies will be required.

Qualifications: A Ph.D degree or the equivalent with experience in mass spectrometry, preferably including lead isotopes at trace levels A knowledge of ore mineralogy is desirable.

Salary: Research Scientiest or Senior Research Scientist. $A14,829 to $A21,543 p.a.

Tenure: Three-year fixed term appointment.

Applications **in duplicate**, stating **full** personal and professional details, the names and addresses of at least two professional referees, and **quoting reference number 602/44**, should reach:

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, London WC2R 3EH by **29th December 1978.**

Applications in U.S.A. and Canada should be sent to:

The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., Washington, D.C., 20036, U.S A

8619(A)

AGRICULTURAL RESEARCH COUNCIL
COMPTON
A CELL BIOLOGIST

is required in the Department of Microbiology. The appointee will be a member of the Scrapie Project Group and required to assist in the development of improved cell culture systems for the scrapie agent. Duties will also include study of the interactions of lymphoreticular cells with the scrapie agent *in vitro* and *in vivo*.

Candidates should have a first or upper second class degree in an appropriate biological subject, preferably including cellular immunology. At least two years' relevant postgraduate research or other approved experience is required for appointment as H.S.O., with four years for S.S.O.

The successful applicant will be appointed either as a Higher Scientific Officer (Salary scale £4,101 to £5,448 per annum) or as a Senior Scientific Officer (Salary scale £5,154 to £6,898 per annum), depending on experience. Non-contributory pension scheme.

Application forms from the Secretary, Institute for Research on Animal Diseases, Compton, Nr Newbury, Berkshire, quoting reference 279. Closing date: December 14, 1978.

8653(A)

UNIVERSITY OF CALIFORNIA
SANTA CRUZ
BOARD OF STUDIES IN ASTRONOMY AND ASTROPHYSICS AND STEVENSON COLLEGE

Applications are invited for the position of.

ASSISTANT PROFESSOR OF ASTROPHYSICS

effective September 1, 1979

Appointee will have graduate and undergraduate teaching and advising responsibilities, will be expected to mount a research program in the Board, and to participate on the academic and intellectual life of the College. Position is in regular tenure-track series, initial appointment is for two years. Applicants must hold Ph.D or expect to receive it by September 1, 1979.

Persons active in theoretical astrophysics with interests in a broad variety of problems, and who would welcome the opportunity of interaction with astrophysicists, physicists and Lick Observatory astronomers, and who have a strong commitment to teaching are preferred. Applications from non-optical, non-IR observational astronomers who have a strong theoretical component to their research interests will also be considered.

Salary, $15,100 to $15,700, nine month basis Applicant should send curriculum vitae, list of publications, statement of research interests and teaching experience, together with names of at least three references who have been asked to submit letters of recommendations directly to us All supporting materials should be sent, timed to arrive not later than January 15, 1979, to:

Dr Robert P. Kraft, Chair
Board of Studies in Astronomy
and Astrophysics
Lick Observatory
University of California
Santa Cruz, CA 95064
(An Affirmation Action/Equal
Opportunity Employer)

8557(A)

UNIVERSITY OF GLASGOW
INSTITUTE OF VIROLOGY
SENIOR LECTURESHIP IN VIROLOGY

Applications are invited for a Senior Lectureship in the Department of Virology at the Institute of Virology Tenable from March 1, 1979, or as soon as possible thereafter. The Institute, which is heavily engaged in biomedical research, comprises both the University Department of Virology and the Medical Research Council Virology Unit, under the direction of Professor J. H. Subak-Sharpe. The successful applicant would have considerable opportunity for interaction and collaborative work with the interdisciplinary research staff. In addition to teaching duties to undergraduates in B.Sc. and M.B., Ch B courses, the applicant would have joint responsibility for the Diagnostic Virology Service to the Western Infirmary and Gartnavel Hospital Applicants should be medically qualified and have suitable specialist experience.

Salary will be according to placement on the University scale for clinical teachers. The maximum on the Senior Lecturers' scale is £12,084

Further particulars may be obtained from the Secretary of the University Court, (Room 18), University of Glasgow, Glasgow G12 8QQ, with whom applications (12 copies), giving the names and addresses of three referees should be lodged on or before January 10, 1979.

In reply please quote Ref. No 4317M. 8608(A)

UNIVERSITY OF RHODESI
DEPARTMENT OF PHYSICS
SENIOR LECTURESHIP/ LECTURESHIP IN PHYSICS

Applications are invited for th post of Senior Lecturer/Lecturer i Physics. Special consideration will b given to applicants with a majo interest in geophysics, or solid stat physics.

Salary scales (Approx. Stg. equiv.) Senior Lecturer £7,483 by £290 t £8,353 by £308 to £9,893.

Lecturer Grade I £6,787 by £255 t £7,807.

Lecturer Grade II £4,190 by £22 to £5,290 by £255 to £6,565.

Both permanent terms and short term one or two year contracts ar offered

Further particulars and applicatio procedure from the Association c Commonwealth Universities (Appts) 36 Gordon Square, London WC1H 0PF.

Closing date for receipt of applica tions **December 31, 1978.**

British subjects considering applyin for posts in Rhodesia are urged t consult the Foreign and Commo wealth Office (telephone 01-233 8727 or their nearest British Consula Office. 8629(A)

THE CALIFORNIA INSTITUTE OF TECHNOLOGY

invites applications for an

ASSISTANT PROFESSORSHI IN RADIO ASTRONOMY

Applicants should have proven r search abilities in the area of radi spectroscopy, as evidenced by publica tions and recommendations fro colleagues. Ability to supervise gradu ate students and to work with pos doctoral fellows is required, teachin ability will be taken into account, ex perience with aperture synthesis tech niques or facility at instrumenta design and development, especially millimetre wavelengths, is desirable.

Facilities are available for bot pencil-beam and interferometric spec troscopy from metre to millimetr wavelengths At the Owens Valle Radio Observatory these include 40m and two 27m antennas, a element interferometer for millimetr wavelengths is under construction, an one 10.4m antenna is presently in use A fourth 10 4m antenna for submilli metre astronomy will be built at high mountain site in 1981 There ar many opportunities for collaboratio within the Pasadena astronomica community.

Applications should include (1) description of educational background (2) a resume of research and teachin experience; (3) a list of all publica tions; (4) copies of all manuscript submitted for publication but not ye published; (5) a statement of curren research interests and future researc plans; (6) a list of at least six astron omers or physicists who can evaluat the applicant's research, including a least three who know the applican personally The applicant should as the latter three to send letters o evaluation directly to Alan T Moffet Director, Owens Valley Radio Ob servatory, 102-24 Caltech, Pasadena CA, 91125 Completed application and letters of evaluation should b received by February, 1979, appoint ment will be made as soon thereafte as circumstances permit ·

Caltech is an Equal Opportunit Employer with an Affirmative Actio Programme We encourage applica tions from qualified women, member of minority groups and persons wit non-disqualifying handicaps 8626(A)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF PHARMACOLOGY

Applications are invited for the post of

LECTURER

available in the above Department. Candidates should have an honours degree and appropriate research experience.

Salary on the scale £3,883 to £7,754 (under review).

Application forms and further particulars available from the Registrar, The University of Leeds, Leeds LS2 9JT, to whom completed applications should be returned by December 18, 1978 quoting reference number 103/2/D.

8634(A)

UNIVERSITY OF EDINBURGH

TECHNICAL OFFICER

Required for the DEPARTMENT OF MOLECULAR BIOLOGY (until 1981 in the first instance) to take charge of the media room and to be responsible, with the help of an assistant, for supplying the Department with growth media. The successful candidate would also supervise the work of the glassware cleaners of the Department. The position would suit a mature person with experience of this work. H.N.C. qualifications preferred. Salary on the scale £3,186 to £3,720 per annum (under review). Ref: NT053. Applications should be sent to the Secretary, Department of Molecular Biology, King's Buildings, Mayfield Road, Edinburgh EH9 3JR. 8639(A)

DUKE UNIVERSITY

DEPARTMENT OF CHEMISTRY seeks appropriate candidates for the Reynolds Industries

PROFESSOR OF CHEMISTRY

an endowed professorship for a distinguished chemist committed to research in any area of chemistry.

Equal Opportunity/Affirmative Action Employer.

Inquiries should be sent to W. R. Krigbaum, Chairman, P. M. Gross Chemical Laboratory, Duke University, Durham, North Carolina 27706, by December 25, 1978.

8659(A)

AGRICULTURAL RESEARCH COUNCIL

INSTITUTE OF ANIMAL PHYSIOLOGY

BABRAHAM, CAMBRIDGE CB2 4AT

SCIENTIFIC OFFICER

required in the Immunology Department to participate in research on the identification and behaviour of lymphocyte subpopulations and lymphoid tissues of farm animals. The work involves a wide range of techniques in haematology, cytology, histochemistry and cellular immunology and experience in these disciplines is desirable. Salary in S.O. scale £2,839 to £4,415 per annum according to qualifications and experience. Applicants should have a pass degree, H.N.D., H.N.C., or equivalent in a relevant biological science and appropriate laboratory experience. Non-contributory superannuation scheme. Application form and further details may be obtained from the Secretary of the Institute. Quote Ref. B.M.R.2. Closing date December 29, 1978.

8596(A)

APPOINTMENTS VACANT

New Fermentation Products

Sefton Park, Bucks

The Glaxo Group of Companies has an impressive history of exploiting fermentation products. Our Fermentation Research Division, situated in pleasant surroundings at Stoke Poges in South Buckinghamshire, has an extensive programme to investigate the biosynthetic potential of a variety of micro-organisms.

We are seeking to fill the following appointments:

Laboratory Head—Microbiologist

This would be suitable for a senior research microbiologist capable of supervising a graduate and technical staff and able to liaise with biochemists, chemists and fermentation technologists within a progressive multi-disciplinary group.

He or she will be responsible for organising and reporting the work of a laboratory and should combine an understanding of secondary metabolite biosynthesis with the ability to apply this knowledge to evaluate the potential of selected organisms for new product formation.

Starting salary in the range of £5,320 p.a. to £7,073 p.a. subject to qualifications and experience. Reference number: 252.

Microbial Geneticist

A newly qualified Ph.D. Microbial Geneticist with a biochemical background is required for work on the genetics of secondary metabolite production in micro-organisms.

Starting salary £4,200 p.a. Reference number: 253.

In addition to the above salary there is a London Allowance of £258.50 per annum, and quarterly and annual bonuses which at present total 10.27% per annum.

Flexible hours of work are in operation and assistance with relocation expenses is available in appropriate cases. When applying for an application form please quote reference numbers as above.

Please apply to:
R. E. Nolan, Personnel Officer
Glaxo-Allenburys Research (Greenford) Ltd.,
Greenford Road, Greenford, Middlesex

8660(A)

Glaxo-Allenburys Research (Greenford) Limited

ANIMAL VIRUS RESEARCH INSTITUTE

APPOINTMENT OF DIRECTOR

Applications are invited for the post of Director of The Animal Virus Research Institute, Pirbright, Surrey, which will become vacant on the retirement of the present Director, Dr J. B. Brooksby, C.B.E., in December 1979. The Institute, which is grant-aided by the Agricultural Research Council, has its own Governing Body and is engaged in research on the virus diseases of animals.

Applicants should have high scientific qualifications and research experience relevant to the Institute's work. The post is graded Chief Scientific Officer and it is the Government's intention that the salary for this grade will be £13,908 per annum from April 1979 and £14,770 from April 1980.

Applications, including the names of three referees, should be received by February 16, 1979 and should be addressed to the Secretary, The Animal Virus Research Institute, Pirbright, Woking, Surrey GU24 0NF, from whom further particulars can be obtained. 8620(A)

UNIVERSITY OF OTAGO

Faculty of Medicine

The Christchurch Clinical School of Medicine

Christchurch, New Zealand

LECTURER OR SENIOR LECTURER IN MEDICINE

Applications are invited from medical graduates for the position of Lecturer or Senior Lecturer in the Department of Medicine in the Christchurch Clinical School of Medicine.

Applicants should have special clinical and research experience in the field of gastroenterology. Specific enquiries should be addressed to Professor D. W. Beaven, Department of Medicine, The Princess Margaret Hospital, Christchurch, New Zealand.

Salary: Lecturer NZ$14,673 to NZ$ 17,700; Senior Lecturer NZ$19,986 to NZ$25,729 per annum. A particularly well qualified applicant could be appointed with the status of Associate Professor NZ$27,026 to NZ$30,728. An allowance for clinical responsibility may be available in the light of status and duties performed.

Further particulars are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close on **February 15 1979.** 8632(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF GENETICS

Applications are invited for the post of postdoctoral Senior Research Assistant to work with Dr P. Strike on an MRC-funded research project studying the enzymology of DNA repair processes in the bacterium *Escherichia coli.* Applicants should have experience in enzyme isolation and/or DNA metabolism. The area being investigated concerns the identification and characterisation of gene products concerned with resistance to DNA damaging agents in bacterial cells.

The project is financed for three years from January 1, 1979, or as soon as possible thereafter.

The initial salary will be £3,883 per annum (under review).

Applications, together with the names of two referees, should be received not later than December 18, 1978, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Reference: RV/405/N. 8606(A)

We have an immediate vacancy in the London office for a

PHYSICAL SCIENTIST

preferably one who has some postdoctoral experience. He or she would be engaged largely in the assessment of scientific manuscripts submitted for publication but would probably also help with other sections of the journal. Experience in the earth, ocean or atmospheric sciences would be an advantage.

Write to:
The Editor
NATURE
MACMILLAN JOURNALS LIMITED
4 Little Essex Street, London WC2R 3LF
enclosing a curriculum vitae and any other material you consider relevant.

8661(A)

DEAN
COLLEGE OF SCIENCE AND MATHEMATICS
UNIVERSITY AT ALBANY

Applications and nominations are invited for the position of Dean of the College of Science and Mathematics, University at Albany. The College consists of Departments of Atmospheric Sciences, Biology, Chemistry, Computer Science, Geological Science, Mathematics and Statistics and Physics. The College includes about 160 faculty, 350 graduate students and 1,100 upper division undergraduates. The Dean is the principal administrative and academic member of the College. His/her essential role is to provide intellectual leadership and direction for the instructional and research programs of the College.

Desired qualifications include an earned doctorate, proven scholarly ability and professional competence, appropriate academic and administrative experience, and the ability to provide creative leadership.

Applications and nominations with resumes should be sent by January 31, 1979, to:

Dr John F. Dewey, Chairman
Dean Search Committee
E.C.C. — LC B31
State University of New York at Albany
1400 Washington Avenue
Albany, NY 12222

The University at Albany is an Equal Opportunity/Affirmative Action employer. Applications from women, minorities, and handicapped are especially welcomed. 8612(A)

UNIVERSITY OF OXFORD
UNIVERSITY LECTURESHIP IN ORGANIC CHEMISTRY

Applications are invited for the above post. Stipend according to age on the scale (under review) £3,883 to £8,257. The successful candidate may be offered a tutorial fellowship by St. Hilda's College (for a woman) or Exeter College (for a man).

Details of the university and college appointments may be obtained from Professor J. E. Baldwin, F.R.S., Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY, to whom completed applications (six typed copies, only one from overseas candidates) should be sent by January 31, 1979. 8588(A)

GRADUATE RESEARCH Assistant required for two years to join team investigating purine metabolism in gout. The work involves the measurement of purine nucleotides and related compounds in food, the effect of these compounds on uric acid metabolism in gouty patients and the monitoring of gout subjects attending the hospital out-patient departments. Good basic practical knowledge of chemistry essential, experience of automated analysis, electrophoresis of H.P.L.C. desirable. Salary not less than £3,409 plus £450 London Allowance. Apply in writing, with full curriculum vitae, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting reference P.M.1. 8648(A)

SOUTHAMPTON HEALTH SERVICES
MEDICAL LABORATORY SCIENTIFIC OFFICER

For the Department of Chemical Pathology and Medicine, Southampton General Hospital, to assist in a two year research study of cyclic nucleotide and phosphodiesterase changes in human liver disease and malignancy. Experience in radioimmunoassay and enzymatic methods would be an advantage. This post would ideally suit a Science Graduate wishing to complete one year as a Junior M.L.S.O. to qualify for state registration. Commencing salary: State registered £3,261 Junior M.L.S.O. £3,015. Application forms and job description from Personnel Department, Tremona Court, 30/32 Tremona Road, Southampton. Telephone Southampton 777222, ext. 3556.

Closing date December 14, 1978.
8595(A)

UNIVERSITY OF GLASGOW
RESEARCH ASSISTANT

Applications are invited for the post of research assistant in the University Department of Pathology, Western Infirmary, Glasgow G11, to undertake studies on immunological aspects of alcoholic liver disease. Applicants should have a good honours degree in one of the biological sciences and may apply to study for a postgraduate degree with this appointment. The appointment will be for three years and will be superannuated.

Salary will be within £3,384 to £3,883 on Range IB of the salary scale for Research and Analogous Staff.

Applications with the names of two referees should be submitted to Professor R. N. M. MacSween at the above address.

In reply please quote Ref. No. 4316M. 8607(A)

UNIVERSITY OF OXFORD
DEPARTMENT OF HUMAN ANATOMY
TECHNICIAN Grade IV
Salary £2,955 to £3,402 p.a.
(under review)

A technician is required to assist with histology and immunocytochemistry and maintenance of a laboratory. Work is in connection with brain research. Knowledge of the cryostat and stereotaxic techniques would be an advantage. The appointment is for a fixed term ending July 31, 1981.

Applications marked 'AC' should be made in writing without delay together with the names and addresses of two referees to The Adminstrator, Department of Human Anatomy, South Parks Road, Oxfrod OX1 3QX.

8650(A)

GUY'S HOSPITAL MEDICAL SCHOOL
Graduate *or* Postdoctoral Research Assistant

required in Physics Department for two years to work with Professor S. J. Wyard on a research project funded by the Medical Research Council, on the deformability of red blood cells.

Initial salary up to £4,407 plus £450 London Allowance, according to qualifications and experience.

Apply in writing, with full curriculum vitae, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref. P.T.1.

8603(A)

SCUOLA NORMALE SUPERIORE
Pisa, Italy

Postdoctoral Position in Theoretical Physics is offered for a period of 10 months from October 1, 1979, for research in one of the fields: astrophysics, plasma physics, particle physics, and field theory.

The total salary attached to this position is seven million Italian lire. Successful candidates may apply for a partial reimbursement of travel expenses.

Applications should be sent by March 31, 1979, to the Director of the Scuola Normale Superiore, Piazza dei Cavalieri, Pisa, Italy, and should enclose a curriculum vitae, a detailed programme of study, and letters from three referees. 8584(A)

IMPERIAL COLLEGE
University of London
LECTURESHIP IN DEPARTMENT OF PHYSICS

Applications are invited for the post of Lectureship to work in the Atmospheric Physics Group, under the general direction of Dr J. S. A. Green. Salary on the scale £3,883 to £7,754 (under review) with superannuation plus London Allowance of £450 per annum.

Applications with curriculum vitae, list of publications and the names of two referees should be addressed to Professor D. J. Bradley, F.R.S., Head of Department, Blackett Laboratory, Imperial College, London SW7, before January 10, 1979. 8613(A)

STRUCTURAL BIOLOGIST

The Department of Biological Sciences, Carnegie-Mellon University, invites applications from individuals whose research interests include the use of biochemical and biophysical techniques to study biological structures. The candidate should have postdoctoral experience. The position is at the assistant or associate professor level. Responsibilities include teaching at the undergraduate and graduate level, and carrying out an active research program. The applicant should forward a **curriculum vitae, three or** more letters of reference, and a brief résumé of research plans to Search Committee (B), Department of Biological Sciences, Carnegie-Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213, U.S.A.

Carnegie-Mellon University is an Equal Opportunity/Affirmative Action Employer. Please respond by December 15, 1978. 8446(A)

GUY'S HOSPITAL MEDICAL SCHOOL
LABORATORY SCIENTIFIC OFFICER

required until September 30, 1980, to work in the Department of Chemical Pathology. Duties will include the *in vitro* cultivation of malaria parasites. Previous experience in tissue culture techniques essential. Salary for qualified Laboratory Scientific Officer not less than £3,261 plus £354 London Weighting.

Apply in writing, stating age and giving details of qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref. C.H.1. 8602(A)

SCIENTIFIC DIRECTOR

Biological Sciences: c. £9,500

Flow Laboratories, a leader in the supply of tissue cultures, reagents, and laboratory equipment, is appointing a European Scientific Director to work from its West of Scotland headquarters. Responsibilities will include contact with the scientific community, research and product development, quality control and the organisation of educational seminars. The Europe wide role will bring the Director into frequent contact with customers throughout the continent. Candidates, probably in their 30s, will be Ph.D.s with detailed up to date experience in virology, microbiology, bacteriology, biology or immunology. They will have an ability to communicate as well with the layman as with the scientist and should see themselves fitting naturally into a strictly commercial environment. Salary is negotiable and together with an annual bonus will be around £9,500. Executive car and other benefits appropriate to this level of seniority. Relocation expenses. Applications with full career details from men and women should be sent in confidence to A. P. Rait, as adviser to the company, at Selection Thomson Ltd., 15 North Claremont Street, Glasgow G3 7NR.

8605(A)

SELECTION ● THOMSON

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

DEPARTMENT OF BIOCHEMISTRY AND CHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

Biophysical chemist required to join research group working on membrane structure and function Initial salary within range £3,909 to £4,655 p.a. plus £450 London Allowance.

Further particulars available from the School Secretary, R.F.H.S.M., 8 Hunter Street, London WC1N 1BP or telephone 01-837 5385, ext. 54 Closing date December 15, 1978 8601(A)

UNIVERSITY OF EAST ANGLIA

Norwich

LECTURESHIP IN ORGANIC CHEMISTRY

Applications are invited for the above Lectureship in the School of Chemical Sciences Salary according to age and experience, will be on the scale £3,883 to £7,754 per annum (under review) plus U.S.S./F.S.S.U. benefits.

Applications (one copy only) which should contain a full curriculum vitae including exact date of birth, together with the names and addresses of three persons to whom reference may be made, should be lodged with the Establishment Officer, University of East Anglia, Norwich NR4 7TJ, from whom further particulars may be obtained (telephone 0603 56161, ext. 2126) by not later than February 1, 1979. No forms of applications are issued In naming three referees you are requested to give only the names of those who can immediately be approached by the University

8590(A)

UNIVERSITY OF BRISTOL

DEPARTMENT OF CHILD HEALTH

RESEARCH ASSISTANT

Post-doctoral scientist or equivalent with experience in electron microscopy, immunology and/or autoradiography to work on immediate hypersensitivity in the lung The position, which is on the salary scale Range 1A for research staff £3,883 to £6,555 per annum (under review) is funded by the Wellcome Trust and is available immediately.

Applications with a curriculum vitae and two referees to Dr F. Carswell, from whom further information is obtainable, Department of Child Health, Children's Hospital, Bristol BS2 8BJ. 8586(A)

WELSH NATIONAL SCHOOL OF MEDICINE

(University of Wales)

LECTURER IN MEDICAL BIOCHEMISTRY

Applications are invited from science graduates with or without a medical qualification, for the post of Lecturer in the Department of Medical Biochemistry at the Medical Teaching Centre, Cardiff. The Department is engaged in teaching both medical and science students and has good facilities for research

Salary either on the scale £4,470 to £7,793 per annum for clinical Lecturers or £3,883 to £7,754 per annum for non-clinical Lecturers (both inclusive or supplements)

Applications should be submitted within four weeks of the appearance of this advertisement to the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN, from whom further particulars may be obtained. 8587(A)

UEA

NORWICH

VICE-CHANCELLOR

The Council and Senate of the University of East Anglia have appointed a Joint Committee to recommend a successor to the Vice-Chancellor, Dr. Frank Thistlethwaite who retires on September 30, 1980.

The Committee invites applications for the post. Persons with appropriate qualification and experience may write privately to the Chairman of the Committee (the Pro-Chancellor and Chairman of the Council, Mr. T. J. A. Colman) care of the Registrar and Secretary, University of East Anglia, if possible no later than the middle of January 1979.

The Committee would also welcome suggestions of the names of persons suitable for consideration and these should similarly be addressed to the Pro-Chancellor.

The Council reserves the right to appoint to the post by invitation.

Information about the University and further particulars of the post may be obtained from the Registrar and Secretary, University of East Anglia, The Registry, Norwich NR4 7TJ.

8598(A)

POSTDOCTORAL RESEARCH POSITION

The Department of Geology, University of Windsor, invites applications for a one-year appointment commencing February-April, 1979. Qualifications: a Ph.D in sedimentology with a basic knowledge of glacial geology, or a Ph.D. in glacial geology with a basic knowledge of sedimentology Stipend $925 per month

Send curriculum vitae and names of three referees to: Dr R. K Jull, Head, Department of Geology, University, Windsor, Ontario, Canada N9B 3P4. 8636(A)

POSITION AVAILABLE

for young Ph.D in biochemistry interested in working on the comparative protein chemistry of the major structural proteins of higher cells Experience in sequence analysis or protein chemistry would be helpful The group is bilingual.

Salary according to experience (not less than DM1,900=~$1,000 or £512 per month tax free).

Application to Klaus Weber, Max Planck Institute for Biophysical Chemistry, D-34 Gottingen, West Germany.

8566(A)

MEDICAL RESEARCH COUNCIL
LABORATORY OF MOLECULAR BIOLOGY
RESEARCH ASSISTANT

required in January 1979 to join a group working on chemical aspects of peptide and oligonucleotide synthesis. Candidates should possess a degree or equivalent qualification in chemistry, biochemistry, or a related science subject. Initial salary on the scale £3,261 (£4,680) with good prospects for promotion to research officer grade and permanency.

Applications giving details of qualifications, experience, and names of two referees, should be sent to me at this address as soon as possible

Dr R. C Sheppard,
MRC Laboratory of Molecular Biology,
The Medical School,
Hills Road,
Cambridge CB2 2QH. 8581(A)

MEDICAL RESEARCH COUNCIL
RADIOBIOLOGY UNIT
CYTOGENETICS

A vacancy exists for a scientist to work in a group investigating the effects of ionising radiation upon chromosomes in a variety of cells and tissues

Applicants should have a good honours degree, and at least three years' post-graduate research experience. They should be conversant with modern cytogenetic techniques and some knowledge of tissue culture is desirable.

Usual Medical Research Council conditions of service Salary according to age and qualifications Applications in writing to: The Administrator, M R C, Radiobiology Unit, Harwell, Didcot, Oxon OX11 0RD. By December 31, 1978 8622(A)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the post-doctoral post of Senior Research Assistant to participate in investigations into mutagenic DNA repair systems in mammalian cells The appointment is funded by the Cancer Research Campaign and is tenable until September 30, 1981

Initial salary within the range £3,384 to £4,382 per annum, depending on age and experience

Applications, together with the names of two referees should be received as soon as possible by The Registrar, The University, P O Box 147, Liverpool L69 3BX, from whom further particulars may be obtained Quote Ref RV/406/N 8623(A)

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Nature

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advertisements

POSITIONS WANTED

PALAEOBOTANIST/ANGIOSPERM TAXONOMIST. Strong background in plant morphology, anatomy, palynology Postdoctoral work in Tertiary palaeobotany, Quaternary palynology Publications. Graduate and undergraduate teaching in North America, Africa (botany/biology) Seeking teaching/research position in Africa, Canada, the Middle East or the United States. Available February, 1979. Contact Box 8664, *Nature*, 3 Dyers Buildings, Holborn EC1N 2NR 8664(B)

COURSES

FEBS ADVANCED COURSES

No. 58, "Biomolecular Electron Microscopy"
Ulm, FRG,
29 March—7 April 1979

The Course, organized by A K Kleinschmidt, is intended to offer information on selected methods of biomolecular structure studies, and laboratory instruction in electron-microscopic methods of macromolecular analysis, to 40 qualified applicants Specialised fields, e.g electron microscopy of nucleic acids, membrane structure, nucleoprotein particles, immunocytochemistry, lipoproteins and proteoglycans, and autoradiographic procedures and image analysis methods will be included to participants' choice in laboratory practical sessions. Course fee, including accommodation and food: approx. DM400 — Applications (by 1 January, 1979) and info· Dr A K Kleinschmidt, University of Ulm, Department of Microbiology, Postfach 4066, 7900 Ulm, FRG

No. 60. "Gene Structure and Formation of RNA of Viruses and Animal Cells"
Israel, 15—21 May 1979

A workshop organized by Y Aloni and co-sponsored by EMBO Fee: $280 — Info: Mr Y Berman, Congress Section, Weizmann Institute of Science, Rehovot, Israel.

No. 62. "Genome Organisation and Expression in Higher Plants"
Edinburgh, Scotland,
11–21 July 1979

A course for up to 125 participants Accommodation including full board, £120 approx. Info. Dr C. Leaver, Dept of Botany, The King's Buildings, Mayfield Road, Edinburgh, EH9 3JH, Scotland.

No. 56. "Membrane Proteins"
Berne, Switzerland,
10–22 September 1979

The course, co-sponsored by the Swiss Committee for Molecular Biology and organized by P Zahler and A Azzi, will consist of 1 week each of lectures and experimental work, and discussions The course will concentrate on specific problems of membrane proteins (solubilization, fractionation, isolation, characterization, reconstitution) Places will be restricted to 80–100 for the lectures and to 40 advanced participants for the experimental part. Fees (including registration, accommodation and food): Sw Fr 700 —for the full course or Sw Fr 340 —for the lecture course only Applications should be addressed before 1 April, 1979 to: Professor P Zahler, Institut fur Biochemie, Universität Bern, Freiestrasse 3, CH-3012 Bern, Switzerland.

No. 57. "Genetic Engineering"
Zagreb, Yugoslavia,
8–24 October 1979

Info: Dr V. Škarić, Institut "Ruder Bošković", Pošt, pret 1016, 41001 Zagreb, Yugoslavia. 8583(D)

UNIVERSITY OF LONDON KING'S COLLEGE
INTERCOLLEGIATE COURSE
M.Sc.
IN
HUMAN AND APPLIED PHYSIOLOGY

A one year course to train graduates in measuring and studying the physiological functioning of man in normal and abnormal environments. The course would equip students to specialise in the applications of physiology to man in industrial, Service and clinical situations and the teaching of human physiology Topics studied include·—basic measurements, life support systems, exercise; locomotor, postural, climatic, high altitude and hyperbaric physiology.

October 1, 1979 to September 30, 1980.

Applicants should normally have a good degree in Physiology or be medically qualified

Further details and application form from Dr S Rowlands, King's College, Strand, LONDON WC2R 2LS, quoting reference 191653/N

Closing date for applications March 31, 1979 8654(D)

N A T.O. ADVANCED STUDIES INSTITUTE— F.E.B.S. WORKSHOP
GENOME ORGANISATION AND EXPRESSION IN PLANTS
July 11–21, 1979, Edinburgh Scotland

This workshop will be concerned with the organisation and expression of the nuclear and organellar genomes, regulation of gene expression, macromolecular properties, biosynthesis and genetic regulation of seed storage proteins; plant viruses, plasmids and viroids

Speakers will include. W. F Thompson (Stanford), J Bedbrook (Cambridge), R. Meagher (Athens, Georgia), R Flavell (Cambridge), P Starlinger (Koln), K Murray (Edinburgh), J. Jendrisak (Minnesota), J. Bishop (Edinburgh), L Dure (Georgia), K Hahlbrock (Freiburg), D. P S Verma (McGill), L N. Vanderhoef (Illinois), G Blobel (Rockerfeller), B Larkins (Purdue), T C Hall (Wisconsin), L Bogorad (Harvard), R. J Ellis (Warwick), J H. Weil (Strasbourg), O. Ciferri (Pavia), F Quetier and F Vedel (Orsay), C J. Leaver (Edinburgh), L Hirth (Strasbourg), Louis Van Vloten-Doting (Leiden), J Schell (Ghent), Mary-Dell Chilton (Seattle), J Kemp (Madison, Wisconsin), H. L Sänger (Giessen), R Dixon (Sussex)

The number of speakers and participants will be strictly limited to 125 and everyone will be expected to attend for the duration of the workshop

Applications with a short curriculum vitae, including present scientific interests and information which might help in their evaluation (e g a letter of recommendation, where appropriate) should be sent in duplicate to Dr C J Leaver, University of Edinburgh, Department of Botany, The King's Buildings, Mayfield Road, Edinburgh EH9 3JH to arrive *not later* than February 14, 1979

Successful applicants will be informed as soon as possible and expected to provide a non-refundable deposit of £25 (which will be deducted from the living expenses of approximately £130). A limited number of grants, to cover partially the living expenses and travel costs, are available but participants are encouraged to cover their expenses from the resources of their own institutions wherever possible. 8592(D)

UNIVERSITY OF SURREY
DEPARTMENT OF MICROBIOLOGY
M.Sc. COURSE IN MEDICAL MICROBIOLOGY

A new, two-year part-time (day release) course in advanced medical microbiology will start in October, 1979 The course is designed mainly for graduates with some experience of working in a medical microbiology laboratory.

For further details, contact the course organiser.

Dr J W Dale,
Department of Microbiology,
University of Surrey,
Guildford, Surrey, OU2 5XH
Tel Guildford 71281, ext 871.
8656(D)

THE UNIVERSITY OF MANCHESTER
Diploma in Bacteriology

Applications are invited for admission to the Diploma in Bacteriology course of one academic year in the session 1979/80 The course is designed to give training and experience in aspects of bacteriology and animal virology which are of general importance in the application of the subject to different specialised fields. Applicants must have qualified in medicine, veterinary medicine, or other branches of science, and have had substantial postgraduate practical experience in bacteriology Medical Research Council awards may be available to suitably qualified British Subjects resident in the United Kingdom Forms of application may be obtained from the Department of Bacteriology and Virology, University of Manchester, Stopford Building, Manchester M13 9PT, and should be returned as soon as possible, and in any case not later than February 1, 1979 8599(D)

STUDENTSHIPS

UNIVERSITY OF SURREY
DEPARTMENT OF BIOCHEMISTRY
S.R C C A S.E. STUDENTSHIP

Applications are invited for a CASE Studentship financed by S.R.C. in collaboration with Shell Toxicology Laboratory, Sittingbourne, Kent, tenable for three years and for work on Molecular studies of the fidelity of DNA repair

Applicants must have upper second or first class honours in Biochemistry or a related subject.

Applications with curriculum vitae and the names and addresses of two referees should be sent to Dr L J King before December 8 For further details, interested applicants are asked to telephone:

Dr King,
Department of Biochemistry,
University of Surrey,
Guildford GU2 5XH
Tel. Guildford (0483) 71281,
Extension 499 8651(F)

MEDICAL RESEARCH COUNCIL
National Institute for Medical Research
DIVISION OF BIOCHEMISTRY

A Medical Research Council Research Studentship is immediately available for work towards a Ph.D. degree with Dr I M Kerr on the mechanism of action of interferon and the control of protein synthesis in animal virus infected cells. The role of interferon-mediated protein kinases and the oligonucleotide effector pppA2'p5'A2'p5'A in these systems and in growth control in normal and transformed cells will be investigated

Applicants must have a first or upper second class honours degree in Biochemistry or a related subject and be available to take up the Studentship almost immediately.

Please apply, including a *curriculum vitae*, as soon as possible to Dr J B Ward, Director of Studies, National Institute for Medical Research, London NW7 1AA (closing date Monday, December 11, 1978) 8642(F)

ASSISTANTSHIP

IMPERIAL COLLEGE
DEPARTMENT OF ZOOLOGY

Applications are invited for a postdoctoral Research Assistantship tenable at the College Field Station for work for three years on a Medical Research Council sponsored project on the sexual development of malarial parasites. The successful applicant will be expected to develop new methods for the culture and purification of the parasite, and to study the biochemistry/physiology of the parasite plasmalemma. Experience in the fields of cell fractionation of membrane chemistry would be an advantage.

Salary in the range £3,883 to £4,631 on the Research and Analogous Staff scale, plus U.S.S.

Applications with the names of two referees in writing by December 15, to Dr R. E. Sinden, Imperial College Field Station, Ashurst Lodge, Ascot, Berks. 8604(P)

THE UNIVERSITY OF MANCHESTER
DEPARTMENT OF CHEMISTRY

Postdoctoral Research Assistantships in Molecular Beam Scattering

Applications are invited for two post-doctoral research assistantships supported by S.R.C. in the research group of Professor R. Grice, to study (a) molecular beam reactive scattering or (b) molecular beam studies of reactions of aeronomic importance. The work involves the measurement of reactive scattering with universal molecular beam machines employing mass spectrometric detection and time-of-flight analysis. Experience in reaction kinetics and vacuum technology would be an advantage.

Initial salaries up to £4,382 p.a. and £4,631 p.a. respectively with appointments for one year in the first instance. Superannuable.

Applications (no forms) to the Registrar, University of Manchester, Manchester M13 9PL, enclosing a curriculum vitae and the names of two referees. Closing date December 20. Quote Ref: 261/78/N. 8609(P)

FELLOWSHIPS

UNIVERSITY COLLEGE DUBLIN
POSTDOCTORAL FELLOWSHIP IN PHYSICAL CHEMISTRY

Applications are invited for a postdoctoral fellowship to investigate the use of circularly polarized light in photochemistry. Applicants should have practical experience in optical and electronic techniques of photochemistry or molecular spectroscopy.

The appointment which is supported by the National Board of Science and Technology, will be made as soon as possible for one year at an initial salary of £3,762 (for newly qualified Ph.D.'s), and may be extended to three years.

Applications, accompanied by a curriculum vitae and the names of two referees, should be sent to:

Dr. Hector Rubalcava,
Department of Chemistry,
University College,
Dublin 4, Ireland. 8637(E)

INVERTEBRATE NEUROBIOLOGY
RESEARCH FELLOWSHIP

available for 2–3 years to study molecular mechanisms involved in control of electrical properties of molluscan neurones. Experience with intracellular recording (preferably in *Aplysia*) required.

CV and three letters of recommendation to: Dr. I. Levitan, Friedrich Miescher-Institut, P.O. Box 273, Ch-4002 Basel, Switzerland. 8643(E)

UNIVERSITY OF WARWICK
POSTDOCTORAL FELLOW IN VIROLOGY

Applications are invited for a Postdoctoral Fellowship with the Virus Research Group in the Department of Biological Sciences. The successful applicant will work in a small group studying the role of defective interfering virus in infections of animals. An interest in fundamental aspects of pathogenesis and a background in virology or immunology is desirable.

The post is for three years, commencing as soon as possible, and is funded by the Medical Research Council. Starting salary will be on the first point of the Research 1A scale, £3,883 (under review). Further details and application forms from the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref. No. 17/A/78.

Closing date for receipt of applications is December 22, 1978. 8647(E)

UNIVERSITY OF EXETER
DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a Postdoctoral Fellowship supported by the Medical Research Council, to work in collaboration with Dr D. H. G. Crout and with Mr R. S. M. Ling, F.R.C.S., of the Princess Elizabeth Orthopaedic Hospital, Exeter, on the metabolism of methyl methacrylate in man. This project is part of a programme in which various aspects of the use of polymethylmethacrylate in Orthopaedic Surgery are under investigation. The research will involve the use of combined gas-chromatography-mass spectrometry. Previous experience of this technique would be desirable though not essential.

The appointment will be for two years, starting as soon as possible. Commencing salary will be £3,883 per annum, rising to £4,133 per annum for the second year of the appointment.

Applications, giving details of age, qualifications and experience, together with the names of two referees should be sent as soon as possible to Dr D. H. G. Crout, Department of Chemistry, Stocker Road, Exeter AX4 4QD. 8621(E)

UNIVERSITY OF EDINBURGH
DEPARTMENT OF VETERINARY PHYSIOLOGY

POSTDOCTORAL RESEARCH FELLOWSHIP
(Scale IA: £3,883 to £6,555 per annum)

Applications are invited for the above post, which is supported by an M.R.C. Programme Grant to Dr A. G. Brown, tenable for five years from January 1, 1979. The Fellow will join a team studying the electrophysiology, ultrastructure and quantative morphology of identified spinal cord neurones. The Fellow will be responsible for computer graphics analysis of neuronal structure. He or she will need to be conversant with computer technology, graphics systems, programming and electronics. An interest or experience in neurobiology would be desirable. A Joyce-Loebl MAGISCAN image analysis system containing a NOVA 3/12 computer will be available.

Applications, including the names and addresses of two referees, should be sent as soon as possible to Dr A. G. Brown, Department of Veterinary Physiology, University of Edinburgh, Summerhall, Edinburgh EH9 1QH. Please quote Reference 5070.

8640(E)

BRITISH DIABETIC ASSOCIATION
LAWRENCE RESEARCH FELLOWSHIP
SUBJECT: DIABETES MELLITUS

ELIGIBILITY: Open only to UK residents. Candidates must be appropriately qualified in medicine or science. VALUE: Appropriate point on NHS or university scale up to registrar or equivalent university) grade. TENABLE: In any approved institution in UK, for 2 years. OTHER INFORMATION: Awarded for independent research. NUMBER: Two offered, normally every two years. (Offered for 1979/80.) PARTICULARS: Available from Secretary-General, British Diabetic Association, 10 Queen Anne Street, London W1M 0BD. Tel: 01-323 1531.

CLOSING DATE: December 20, 1978. 8657(E)

THE ROYAL SOCIETY
BRUNO MENDEL FELLOWSHIPS

Applications are invited for Bruno Mendel Fellowships for one year from October 1, 1979, renewable for one and exceptionally two additional years, from postgraduate candidates normally resident in the United Kingdom, The Netherlands or Israel, to carry out research in medicine or the biomedical sciences at a university, medical research centre or medical school. Stipends will normally be at the level of a junior research fellowship appointment in the United Kingdom; additional allowances for travel and superannuation may be provided as appropriate.

Application forms, receivable by January 15, 1979, and regulations (revised in May 1976) are available from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG. 8611(E)

UNIVERSITY OF SOUTHAMPTON
SCHOOL OF BIOCHEMICAL AND PHYSIOLOGICAL SCIENCES

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a Wellcome Trust supported appointment for two years as a postdoctoral research fellow investigating the causes of *oedema in pregnancy*. Candidates should have experience and an interest in endocrinology, salt and water balance, thirst and salt appetite or cardiovascular physiology. The successful applicant will enjoy working with animals and will be able to initiate and conduct his/her own research, within the limits defined by the grant.

The appointment is initially for one year, renewable for a further year, and can commence in January 1979.

Salary: £3,883 per annum for Year 1; £4,133 per annum for Year 2 (under review). U.S.S. benefits. Please write for further details to Dr P. Burstyn, School of Biochemical and Physiological Sciences, Boldrewood, Southampton.

Applications giving date of birth, details of qualifications and experience and the names and addresses of two referees, should be sent to Mrs. P. Vaughan-Smith, The University, Southampton SO9 5NH by December 15, 1978. Please quote ref: 1041/R/N.

8591(E)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF AERONAUTICS AND ASTRONAUTICS

RESEARCH FELLOWSHIPS *or* ASSISTANTSHIP IN COMBUSTION

Applications for the above post are invited from suitably qualified engineers or scientists to take part in a fundamental, experimental and theoretical research programme, funded by the United States Air Force, to assist development of new types of combustion chamber for propulsion systems. The appointment would be for one year in the first instance but it is hoped that the research programme will continue for a further period.

Salary will be within range 1A or 1B up to £4,631 per annum (under review) plus U.S.S. benefits.

Applications giving a brief curriculum vitae and the names of two referees should be sent by December 8 to D. A. S. Copland, The University, Southampton SO9 5NH, from whom further particulars may be obtained. Please quote reference 176/R/NSc. 8600(E)

CONFERENCES

24th OHOLO BIOLOGICAL CONFERENCE
on
NEUROACTIVE COMPOUNDS AND THEIR CELL RECEPTORS
on
1st to 4th APRIL, 1979
at
ZICHRON YA'ACOV
ISRAEL

Applications and further information from: Mrs Ariela Snir, Secretary to the Oholo Conference, Israel Institute for Biological Research, P.O. Box 19, Ness-Ziona, Israel.

Invited Papers Only.

8662(C)

INTERNATIONAL CONFERENCE
and POSTER PRESENTATION on
ELECTROKINETIC PROPERTIES AND FUNCTIONS OF BIOPARTICLES
MARTINSRIED near MUNICH, GERMANY
OCTOBER 13–19, 1979

Topics: Dynamics of cell membranes; Surface proteins: changes during cell growth and transformation; Assembly and turnover of subcellular structures; Analytical cell electrophoresis and its application in clinical diagnosis.

Information: Professor Dr Kurt Hannig
Max-Planck-Institut für Biochemie
8033 Martinsried, Germany 8658(C)

A MEETING ON RECOMBINANT DNA

Organized by the Committee on Genetic Experimentation (COGENE), of the International Council of Scientific Unions and the Royal Society of London and will be held at Wye College Kent from 1-4 April 1979. The purpose of the meeting is to review the current status of recombinant DNA research and technology and to discuss matters of concern such as support for the research, its regulation through guidelines and legislation, and the eventual practical application of the technology.

Representatives of national recombinant DNA committees and other involved national and international organizations are being invited but a small number of places are being reserved for general applicants.

The registration fee for the Conference is US $80 or £40.
The charge for accommodation and full board is US $120 or £60.

Those wishing to participate should write for a registration form and further details to The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, England.

8582(C)

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(1) Gupta, R., and Randerath, K. (1977). Nucleic Acids Res. 4, 3441
(2) Simoncsits, A., Brownlee, G. G., Brown, R. S., Rubin, J. R., and Guilley, H. (1977). Nature 269, 833.

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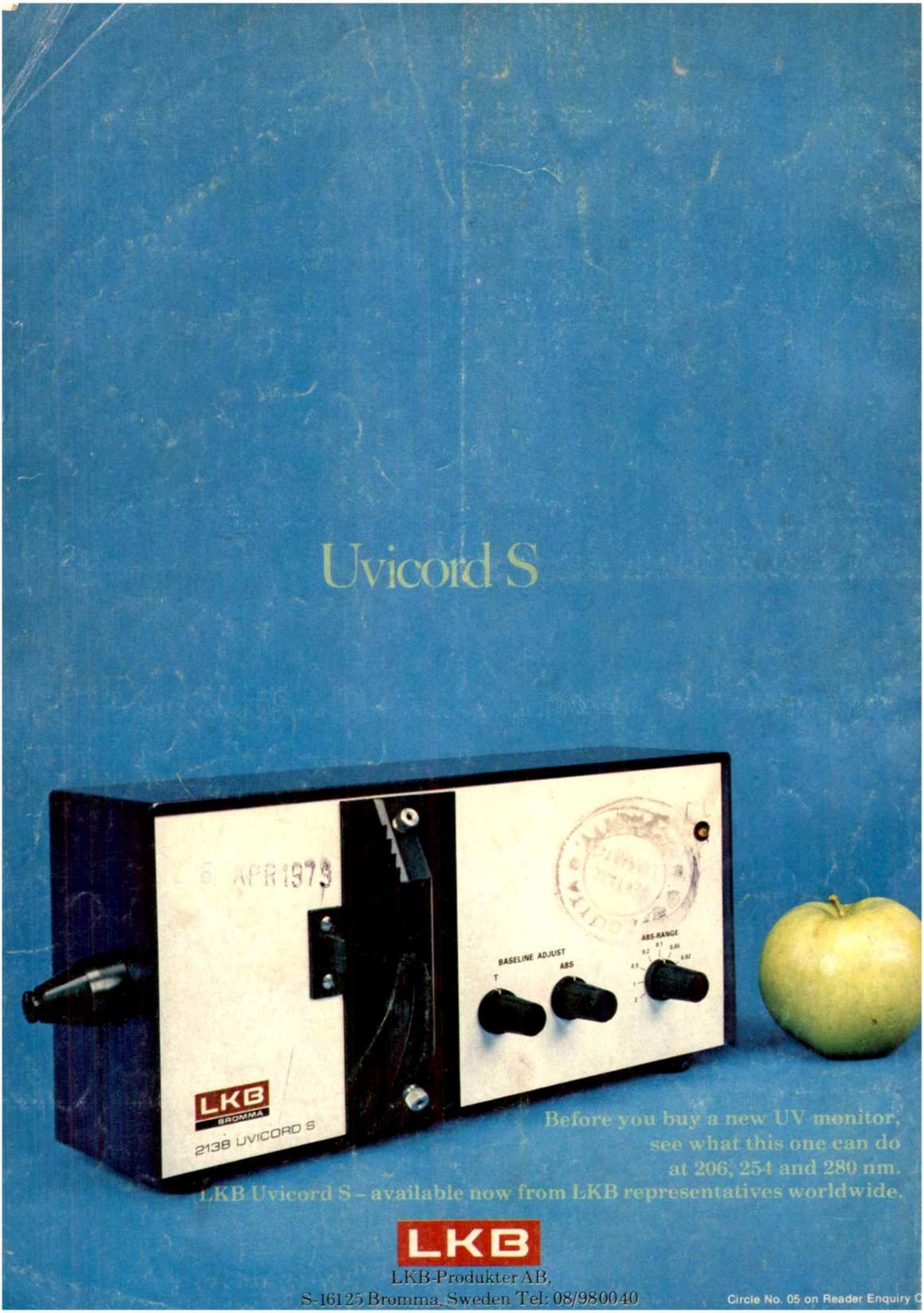

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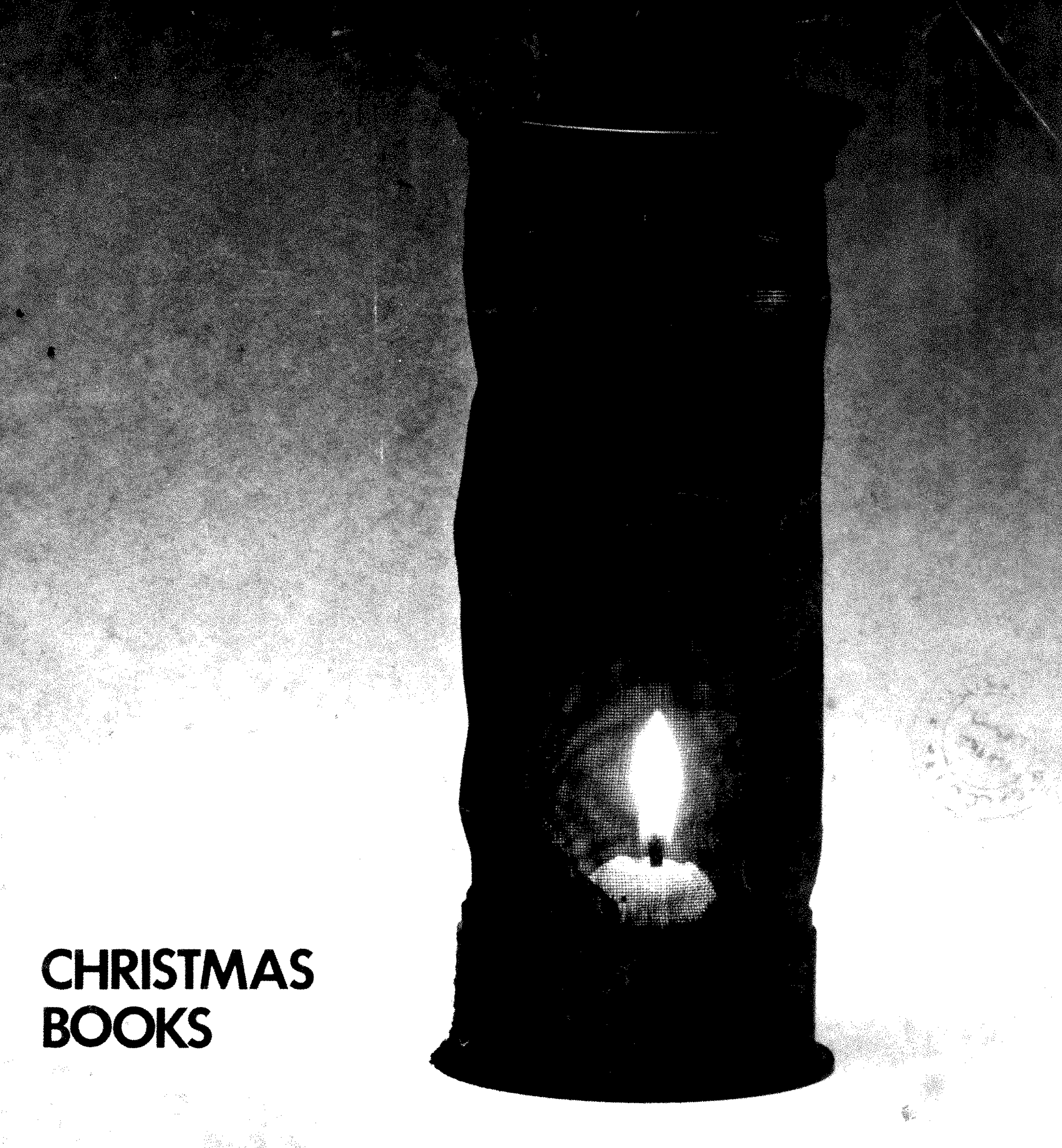

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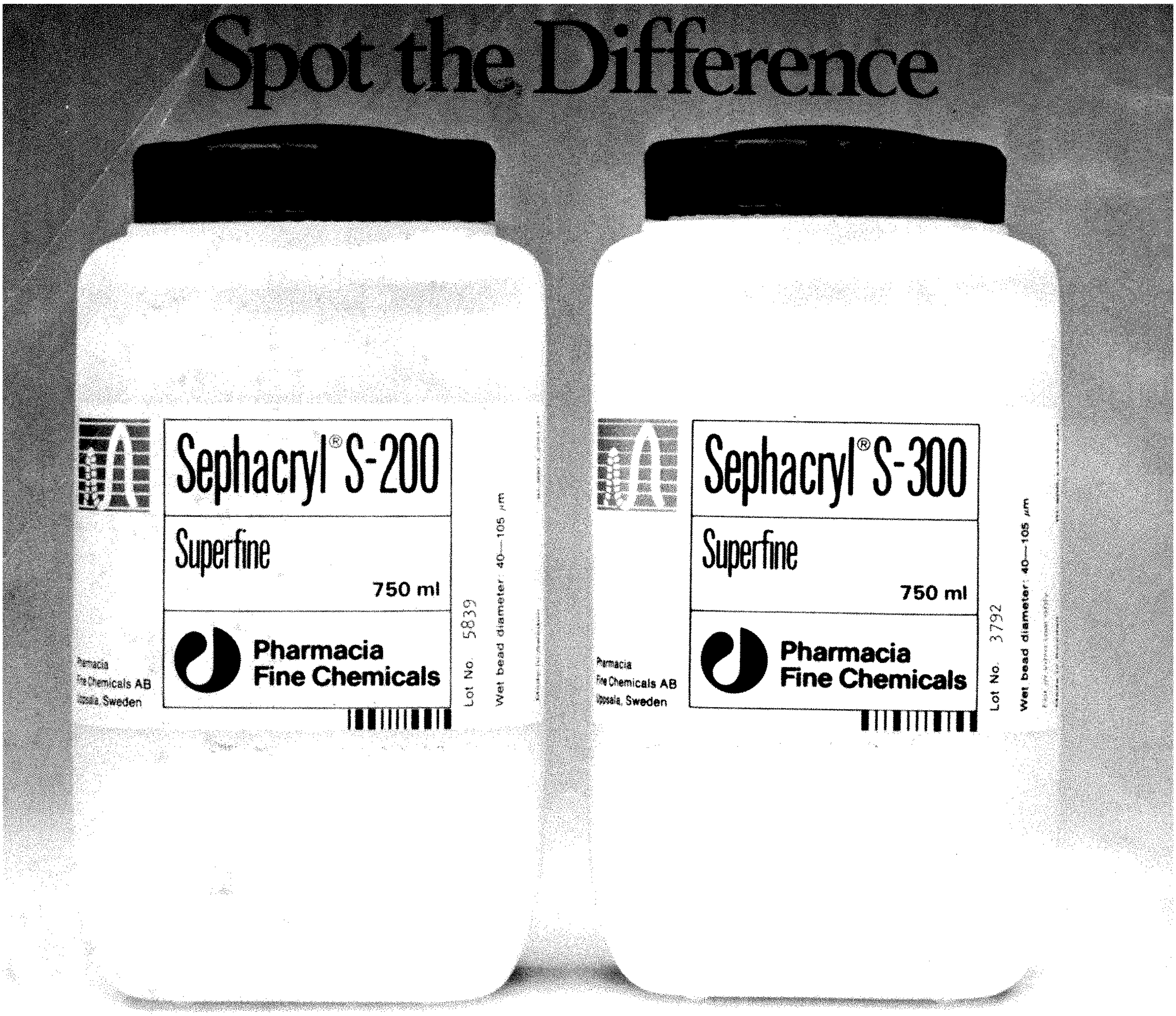

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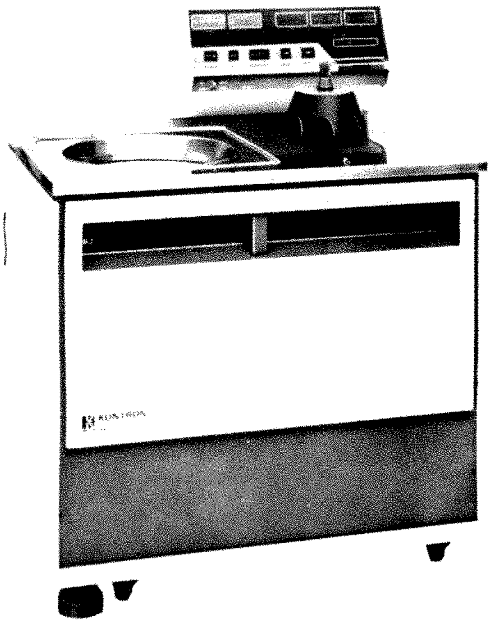

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7 December 1978

Science has a word for it

" I'M SORRY, but arranging for you to see Dr Smith in the next few weeks is going to be very difficult; he's **delocalised** between London and Geneva". So said a scientist on the phone to us recently. The thought of the fairly ample Dr Smith being a mere probability density function and subjected to an uncertainty principle started a train of thought about how scientists could enrich or befoul the English language.

Some jargon has, of course, already made the jump, sorry the transition, from the laboratory to everyday use—for instance half-life. *Fall-out*, an undesirable consequence in its scientific context which became a desirable one in its general usage has got even further—to retirement. But there are riches untouched as yet, and others not well enough known. Here are a few examples.

world-line: 'If only I'd known you were in London yesterday, we could surely have made our world-lines intersect'. One way to have done this, of course, would have been to settle on a **time** and **space window.** That would have put up the **collision cross-section** by **orders of magnitude.**

hysteritic: Suitable as a descrption of management or bureaucracy. You push in one direction and you encounter resistance and delay; you reverse your direction of attack and lo and behold the self-same resistance and delay meets you.

boundary conditions: When you are looking around for a new job you specify certain requirements—rural environment, good schools in the neighbourhood, no overtime, easy access to London . . . In short, boundary conditions. A search through the classified advertisements then yields a set of suitable jobs consistent with them; presumably these are called **eigenfunctions.** The salaries, or **eigenvalues,** often bear a strong similarity to each other. This is hardly surprising given the nature of the world. But scientists even have a word for that: **degeneracy.**

boeing: Not strictly a scientific word, of course, but refers to the behaviour of many scientists. Many laboratories have one person who is always flying off to conferences. He is said to be boeing around. I bo, you bo, he boes; though of course you and I don't do it.

orthogonal: A slightly derogatory term. 'His interests are pretty orthogonal to mine' indicates that of all the wealth of my interests, he can't find a thing in common with me. Other stronger insults are to imply that a colleague has **limited bandwidth** or, what comes to the same thing, **high-Q**—a narrow view of the world and an ability to go on and on when given the chance. Nor is calling someone **non-linear** polite. It suggests that under pressure they either fade or over-react.

deconvolution: Ask a simple question and some people, with complex **transfer functions,** convert your **input** into highly convoluted **output.** Deconvolution is simply finding out whether they said yes or no. You may also have a **signal-to-noise ratio** problem to contend with.

perturbation: invariably associated with an order. A **first-order** perturbation is very large indeed, such as getting married, having children, changing jobs. A **second-order** perturbation is trifling; for instance picking up a colleague on the way to work, answering the phone for him when he's out of the room.

tunnelling: Every graduate physicist knows that there isn't a potential barrier so high that it can't be passed through by hanging around outside it long enough. So to 'tunnel' is to get where you don't belong simply by persistence. Hence 'I see that at long last Jones has tunnelled his way into The Royal Society, the professorship etc. It sounds quite polite, even creditable, and suggests a certain amount of beavering; preferable to talking of Buggins' turn.

Do not confuse tunnelling with the ability of Hungarians to enter a revolving door behind you and come out ahead of you. This is a genuine and quite simple skill, and science has a word for it, too; **spin-flipping.** Those subjected to it have been known to remark 'What's happening to this spin-flipping door?'

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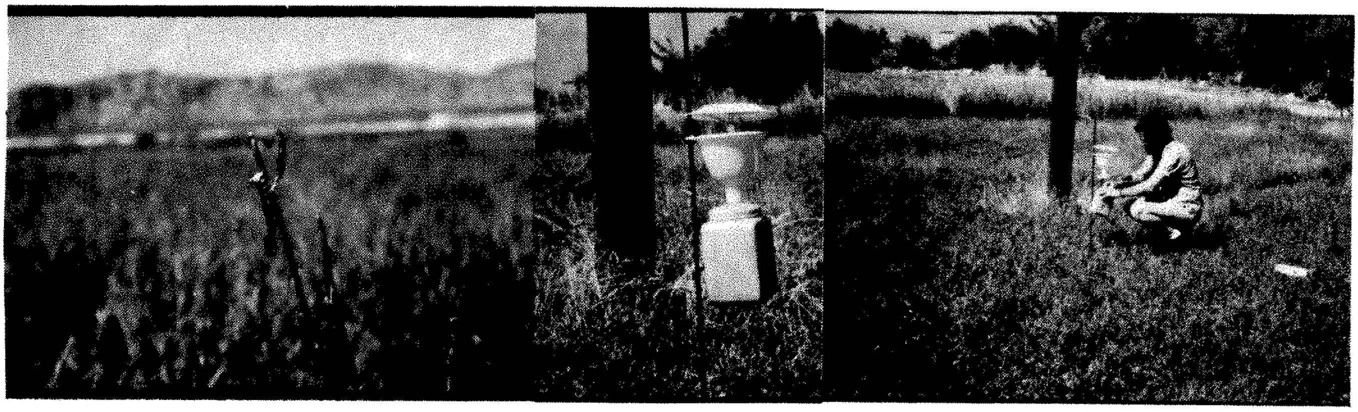

UK investigates virus insecticides

Pathogenic insect viruses may be a safe and effective alternative to chemical insecticides. The Unit of Invertebrate Virology at Oxford and the Centre for Overseas Pest Research in London are already conducting field trials with one class of virus: Baculoviruses. **A. J. McClelland and Peter Collins** report

THE EARTH is dominated by insects: they are present in staggering numbers (a billion billion, according to one estimate) and they are found in every habitable part of the globe. There is a greater diversity of insects than of any other group of animals, and they occupy many different, but essential, ecological niches. Fortunately, only a tiny minority, about 0.1% of species, are harmful to humans. This, though, represents about 3,000 insect species which can cause an immense amount of damage. Insect pests are vectors for several human diseases and also responsible for reducing available food supplies in the world by about 30%. Controlling them is thus of paramount importance in a world where food supplies are often unable to meet human needs.

For the past 40 years, chemical insecticides have been the primary method of controlling insect populations. This is because chemical insecticides were cheap to manufacture, easy to apply and highly effective. By comparison, biological methods of control appeared to be ineffective and unpredictable. But it is now realised that chemical insecticides are not the panacea they were once thought to be. First, the target insects have a remarkable ability to develop pesticide-resistance; second, chemical agents are not species specific and thus kill many other (often beneficial) insects in addition to the ones they were aimed at. Hence the increasing interest in the use of natural insect pathogens—parasites, bacteria, and viruses.

Of all the known insect pathogens, viruses appear to have the greatest potential for controlling insect pests. One group of viruses in particular—the Baculoviruses—seems to be especially suited to this purpose since natural epizootics of these viruses provide ample evidence of their lethality for the larvae of their host species. However, attempts to use Baculoviruses to control insect populations have yielded mixed results. For example, the corn earworm (*Heliothis zea*) has been effectively controlled by viruses in the USA, but attempts to control the East African armyworm (*Spodoptera exempta*) have not been as successful. Those equivocal results were largely a consequence of the crude and haphazard methods that were employed. In some instances infected larvae were simply mixed with water, mashed up and used as a spray. Little research was done on the nature of the viruses, the dose required, or the possible side effects of the viruses on the environment.

The effective use of biological methods of control depends on a substantial knowledge of both the pathogen and the host. The Natural Environment Research Council's Unit of Invertebrate Virology (UIV) was established in Oxford under the direction of Dr T. W. Tinsley in order to obtain more knowledge about insect viruses. Tinsley is collaborating with Dr P. Haskell at the Centre for Overseas Pest Research (COPR) on a research programme to study Baculoviruses and conduct field trials to determine the efficacy of these viruses as insecticides. The aim is to establish the conditions under which the virological control of insect populations would be effective, reliable and safe, and thus provide an alternative to the existing methods of controlling insects with chemicals. Tinsley and Haskell's work reflects a widespread revival of interest in the concept of 'integrated control'.

Integrated control is the manipulation of the insect's ecosystem so as to reduce the population of a pest insect species with minimal disruption of the environment. The technique relies heavily on biological pathogens that are specific for the pest species, but does not preclude the limited use of other agents including certain non-persistent chemical pesticides. However, before viral pathogens can be used in such a programme, they must be fully characterised. To quote Dr Tinsley, "the indiscriminate release of replicating viruses into the environment is as reprehensible as the indiscriminate release of chemicals".

The UIV/COPR programme is divided into the following four stages:

- The development of methods for identifying, purifying and isolating viruses pathogenic to insects.
- Investigation of the possibility that a useful virus could infect species of insect other than the target species, or even vertebrates, particularly mammals.
- Laboratory testing of the efficacy of the purified viruses as insecticides.
- Field trials to confirm the results of laboratory testing and to determine the dose that must be applied to obtain high rates of kill.

According to Dr D. Knudson of the Yale School of Public Epidemiology, the UIV programme is unique in that virological research, research into the potential environmental effects of insect viruses, and field trials to determine the best way of using the viruses as insecticides are combined into a unified programme. The research at UIV is concerned primarily, but not solely, with Baculoviruses isolated from *Neodiprion sertifer* (the pine sawfly) and from four species of the genus *Spodoptera*, namely *S. exempta* (the East African armyworm), *S. littoralis* (the Mediterranean cotton worm), *S. frugiperda* (the US fall armyworm) and *S. exigua* (the US beet armyworm), which are major pests in the areas in which they occur.

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●Left: Dying larvae of Spodoptera littoralis hanging from lucerne stems. Centre: Pheromone trap used during field trials to keep a check on the moth population and, thereby, the extent of virus infection. Right: Derrick McKinley of COPR inspects the trap.

Before any Baculovirus can be released into the environment, its ability to infect other species of insect and vertebrates, such as birds (which feed off the insects), as well as the ecological problems that may arise from the collapse of an insect population must be studied. Fully half the research effort at UIV over the last five years has been devoted to ensuring that Baculovirus insecticides are safe and will not harm the environment. To date no evidence of any potential harmful effects has been found. However, as a result of the research that has been done by the UIV, and others, quite a bit is now known about Baculoviruses, their host range, the way they infect their host and the effect they are likely to have on the environment.

It is known, for example, that Baculoviruses contain DNA and are large, rod shaped particles (about 100 mm by 300 nm). Experimental studies have shown that the majority of Baculoviruses are species-specific, although a few types have a slightly wider host range. However, all Baculoviruses are restricted to invertebrates and, as a group, they appear to have no chemical, physical or biological properties in common with any other class of virus.

The ability of the Baculoviruses to infect mammalian cells is being tested at Porton, the British laboratory that was originally established to do research into defence against 'germ warfare'. The results so far indicate that Baculoviruses do not infect mammalian cells and do not cause disease in animals. However, the details of the viral replication cycle are not yet fully understood and there is a possibility that Baculoviruses could induce the expression of a latent virus, for example an oncornavirus, in a mammalian species. That seems to be a remote possibility, in view of the restricted host range of Baculoviruses, and in view of the large number of experiments in which no covert Baculovirus infections in tissue culture grown mammalian cells could be detected, but it is now being investigated.

Immunological techniques for identifying and distinguishing among Baculoviruses from different insects have recently been developed by the UIV. This is an important development, since it enables the viruses to be monitored after they have been released into the environment. Thus, if the population of a species of insect, other than the target species, collapses after the release of a Baculovirus it can be quickly determined if the virus that was released is responsible or not. The goal of any insect control programme is, of course, to reduce severely or cause the collapse of an insect population. The environmental effects of inducing such a collapse with a Baculovirus insecticide can only be estimated by examining the effects of natural epizootics of these viruses.

Two spectacular examples of Baculovirus epizootics have occurred—one in Canada during the late 1930s and one in Wales in the mid-1970s. In both cases there appeared to be no measureable effects, on, for example, the birds that normally fed on the target insects—they simply changed their diet. It is thought, therefore, that the environmental perturbations caused by species specific virus insecticides will be limited.

A substantial proportion of the research effort at UIV is concerned with the way in which Baculoviruses are disseminated under natural conditions, how they infect their host and how the natural infections are controlled. A characteristic feature of Baculoviruses that has been found to be important in their dissemination, and which also reduces their ability to infect vertebrates, is that they form polyhedral inclusion bodies which contain virus particles and which are highly resistant to disruption. The outer crystalline protein matrix of the inclusion bodies can only be disrupted at a very high pH (above 10) such as that which exists in the digestive tract of larvae. Insects appear to become infected with Baculoviruses by ingesting the virus and/or virus inclusion bodies with their food. However, the inclusion bodies cannot be disrupted by the human digestive tract (thus protecting humans from exposure to infectious virus) or by the digestive tract of birds. Inclusion bodies thus pass through the gut of birds that have fed on infected larvae and are disseminated in the environment.

The stability of the inclusion body has important implications for the way in which Baculovirus insecticides could be used. Since the infectivity of the virus inside the tough outer matrix is preserved it could mean that the virus could be readily stored as a dry powder or aqueous solution which need only be applied infrequently. On the other hand, it may be necessary to create an artificial epizootic every year. The only way to resolve this type of problem is to conduct extensive field trials.

Both the UIV and COPR have started field trials with Baculovirus insecticides. In Britain, the UIV has received clearance from the Ministry of Agriculture's Pesticide Safety Precaution Scheme to test the virus isolated from the pine sawfly (*N. sertifer*) to see if it can be used to control this forest pest. In the COPR programme, preliminary trials using the *S. littoralis* virus have been done in Crete and a more extensive study of this virus will be started soon in the Egyptian Fayoum. The preliminary results of these trials are very encouraging. For example, it has been found that spraying viruses from a low flying helicopter is an effective method of controlling *N. sertifer* — probably because the downdraught from the helicopter helps to distribute the virus.

In both the UIV and COPR trials novel ways of applying the viruses are being tested and, in keeping with the philosophy of integrated control, different combinations of agents are, or will be, used. An example of this is the proposed use of pheromones in Egypt by the COPR team. Pheromones will be used to attract male adult cotton worm moths (*S. littoralis*) into traps containing the virus insecticide. It is hoped that these moths will transmit the virus to the females during copulation who, in turn, will contaminate the outer shell of their eggs with the virus during oviposition. Since the first thing the larvae do after hatching is to eat their own eggshells, they will immediately become infected and die before they can do much damage to the crop.

The same rationale lies behind the concept of prophylactic spraying, enunciated by Dr Tinsley at the UIV. In this approach, low doses of viruses are sprayed before the eggs hatch. The advantages of this approach are first, that the larvae are killed soon after hatching and before they damage the plants or crops they normally feed on, and second, that since only low doses are needed to kill the hatching larvae, any possibility of upsetting the environment is minimised. The methods being tested in the field trials show clearly that the knowledge that has been obtained of the biology of the Baculoviruses and their insect hosts, is being put to good use.

Virus insecticides have the potential of being able to control many different species of insect. For the first time, therefore, we can envisage an alternative to the widespread use of chemical insecticides—and this at a time when chemical insecticides are becoming more and more expensive to manufacture.

In contrast, virus insecticides should be relatively cheap to produce, and should be within the capability of countries that do not have large petrochemical industries. Since a large proportion of the agricultural crops grown in the Third World are destroyed by insect pests, this research is potentially of enormous benefit to underdeveloped countries. ☐

US has doubts on anti-proliferation proposals

PROPOSALS presented jointly by the UK Atomic Energy Authority (UKAEA) and the US Electric Power Institute (EPRI) for reducing the proliferation dangers associated with nuclear power plans have received a less-than-enthusiastic reception from a number of groups concerned with US nuclear policy.

The proposals were announced at a meeting in Washington last February by Dr Walter Marshall, deputy chairman of the UKAEA, and Dr Chauncey Starr, president of EPRI. They involve a new system for reprocessing spent reactor fuel known as CIVEX which, it is claimed, makes the diversion of nuclear fuel virtually impossible by ensuring that pure plutonium is not accessible at any point of the cycle.

The proposed system is now being looked at closely as part of the International Nuclear Fuel Cycle Evaluation (INFCE) study, set up last year largely at the suggestion of President Carter. It has already been studied by a number of organisations in Washington, and received a variety of responses.

In the report of a study group set up to assess the CIVEX concept, the industry-based Atomic Industry Forum said that it found the concept to be "technically feasible" and "worthy of further development" and that it represents a considerable advance in answering fears that plutonium could be diverted from the fast-breeder cycle.

However the study group points out that there are a number of technical and economic questions that must be resolved before CIVEX's true potential can be realised. And it adds: "A breeder cycle embodying the CIVEX concept does not significantly increase diversion risk in comparison with no breeders at all. Other routes for obtaining fissile material exist independent of the breeder and dominate the diversion risk in either case".

Considerably stronger criticism of the CIVEX concept was made by one of the five members of the Nuclear Regulatory Commission (NRC), Mr Victor Gilinsky, speaking at a recent conference in London sponsored jointly by the AIF and the British Nuclear Forum.

"It is difficult to take CIVEX seriously as an answer to the manifest danger of dispersing plutonium in weapons usable form, a problem we will face, according to Judge Parker, ten years from now; there is no conceivable way this scheme can be implemented within the period Judge Parker has allowed us," he said.

Drawing on internal studies that have been conducted by NRC staff, Mr Gilinski, who was previously director of applied science and technology with the Rand Corporation, said that CIVEX fuel remained radioactive long enough to be self-protective for a "fleeting period of time" compared to spent fuel. Its radioactivity was so diminished a few years after leaving the reactor that it could not provide the protection for which it was designed, he said.

"Since most spent fuel reprocessed in this century will have cooled for longer than that, the CIVEX process cannot contribute to the solution of the problems we must worry about now," Dr Giliknsky said. The "abortive flurry" over CIVEX, underlined the importance of attacking the problem of plutonium return now.

Equally harsh words have come from public interest groups. An evaluation carried out by Jim Cubie of New Directions and Tom Cochran of the National Resources Defense Council comes to the conclusion that CIVEX is "much less promising than at first glance", and that rather than being proliferation proof, it at best increases proliferation *resistance*.

"Even though it is intended for use in the next mid-century, because CIVEX has been claimed to make reprocessing acceptable, it will be used as an excuse for undertaking new plutonium reprocessing ventures in the next few decades and thus spread nuclear weapons capabilities," the two authors say.

David Dickson

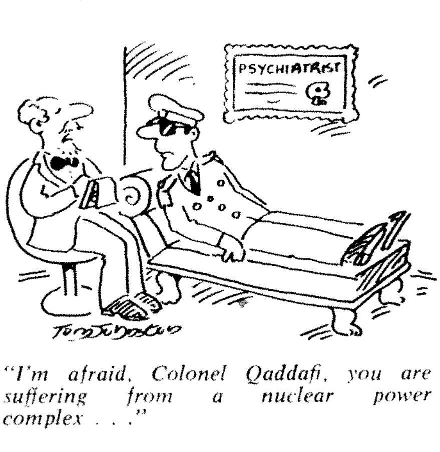
Libya bidding to join nuclear club, scientists warn

LIBYA is actively seeking nuclear weapons even though it signed the nuclear non-proliferation treaty three years ago, warns the Federation of American Scientists. The FAS is urging the Soviet Union to cancel an agreement to sell Libya a 400-megawatt nuclear power complex.

Jeremy Stone, director of the Washington-based federation, said that Ahmed al-Shahati, who is head of the foreign liaison office of the People's General Congress, told him openly that Libya is still trying to obtain an atomic bomb. "Shahati made no bones about it, saying they would seek all weapons with which to defend themselves," Mr Stone said of his conversation in Tripoli in October. "To be sure I understood, I asked again: were they seeking to maintain the right to get a bomb or actually trying to get the bomb itself? It was the latter."

The Soviet Union, whose policy is only to sell nuclear technology to countries that have ratified the non-proliferation treaty, announced the Libyan contract in October. The Russians are currently negotiating safeguards with the International Atomic Energy Agency to prevent nuclear fuel being diverted from the Libyan reactor to make weapons. Professor George Rathjens of the Massachusetts Institute of Technology, who is chairman of the FAS, estimated that the Libyan plant would produce enough fissionable material to produce "a couple of dozen" bombs a year.

In a letter to Anatoliy Dobrynin, the Soviet ambassador in Washington, Mr Stone asked: "Can the Soviet government rely upon the Libyan government to comply with the terms of the future IAEA safeguards agreement if Libya cannot be relied upon to comply with the treaty itself?"



"I'm afraid, Colonel Qaddafi, you are suffering from a nuclear power complex . . ."

Libya's nuclear ambitions were well known in the early 1970s, when its leader Muammar al-Qaddafi reportedly tried to buy nuclear weapons from China and later said he wanted to purchase the bomb from anyone who would sell it to him. Mr Stone said these attitudes did not change when Libya cynically ratified the treaty to become eligible for the Soviet reactor.

The FAS has also written to President Carter, asking him to take the matter up with the Russians and raising the possibility of international sanctions against Libya. There is not much the US can do on its own, Mr Stone said, apart from sending home the 2,000 Libyan students in American colleges and universities (10% of whom are studying nuclear science.)

The federation also named four other "potential false adherents" to the non-proliferation treaty: Taiwan, South Korea, Iraq and Iran. But Mr Stone did not claim to have any direct evidence that any of them was blatantly seeking nuclear weapons like Libya. □

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Czech chartists claim two died in nuclear accident

CHARTER-77, the Czechoslovak human rights movement, last week entered the nuclear debate, with an exposé of conditions at the Jaslovske Bohunice power station. According to Document 22 of the Charter movement (distributed abroad by the Palach press), employees at the power station have been compelled (under threat of loss of premium payments) to expose themselves to radiation levels considerably above the safety standards, while, in the course of the last three years, two serious accidents, one of them causing the death of two workers, have taken place at the station. Indeed, claim the Chartists, since the second accident in February, 1977, the station is still "temporarily" closed.

The Jaslovske Bohunice power station has a long and chequered history. It was constructed under the terms of the Soviet-Czechoslovak general agreement for nuclear energy cooperation of April 1955. In 1956, it was predicted that the station would be commissioned in "about 1962"—in fact, it went critical in 1972 and became fully operational the following year.

According to Dr Frantisek Janouch, who at that time was working at the Czechoslovak Institute of Nuclear Research, and who took part in the many professional and organisational discussions relating to the construction of the reactor, the project was essentially a Soviet proposal and was carried out under Soviet supervision. The station uses a 110 MW gas-cooled heavy-water reactor. According to Dr Janouch, the Russians wanted to see if it was possible to construct an effective reactor of this type—leaving the details to be worked out by the Czechs and done at Czech cost.

In 1969, before the Jaslovske Bohunice station, known as the A-1, was completed, the Czechoslovak nuclear energy industry was switched, following a Soviet "recommendation", to light-water pressure reactors of the VVER type, for which the Soviet Union would supply "a substantial part of the main equipment". Whether this change of plan affected the final stages of work on the A-1 is not clear; it appears, however, from the Chartists' report that the projected automatic system for mounting new fuel elements was never brought into operation, and the mounting was done manually. Workers on the reactor were, says the report, "under psychological stress", often working a 16-hour shift instead of the six hours or less customary in "developed" countries. On 5 January, 1976, an error occurred in the mounting process. The element shot out of the reactor, under a pressure of 60 atmospheres together with a large quantity of radioactive CO_2. Since the emergency gas-traps and filters were insufficient for an accident of this magnitude, radioactive gas escaped into the atmosphere. In the area of the accident, emergency evacuation plans went into operation; unfortunately, one escape door had been locked, apparently to reduce petty thefts, and two workers were suffocated.

Some six weeks later, however, disaster struck again (according to the Chartists). During the mounting of new fuel cells, the primary circuit overheated, the air-tight seal of the steam generator ruptured, and, as a result, the primary circuit, part of the secondary circuit and the working area all became contaminated. Radioactive material entered the drainage system of the plant and a stream in the vicinity has since had to be "fenced off" as contaminated

During the repair work to the reactor, says the Chartists' document, safety levels of radiation were increasingly ignored, in an attempt to expedite the work.

The anonymous authors of the document urge nothing less than an open discussion and local referenda as to whether nuclear power stations should be constructed at all. This is all the more remarkable since uranium is Czechoslovakia's sole native energy source of any magnitude, and current plans evisage a nuclear expansion of 10,280 MW over the next 15 years, so that by 1990 over 30% of the installed generating capacity will be nuclear.

With such a major commitment to nuclear energy, the reaction of the Czechoslovak authorities to the report is predictably to deny everything. No such accidents occurred, they say, and even if they had occurred, they were under no obligation to make any public announcement.

Vera Rich

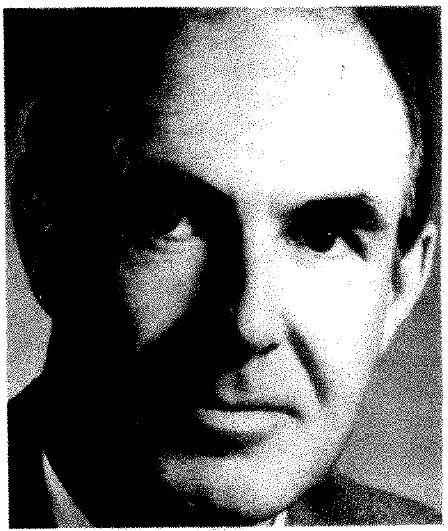
Canadian scientists confused by government moves on R & D funding

LAST week the Canadian Government appointed a new Minister of State for Science and Technology, Mr Alastair Gillespie. A brief occupant of the post when it was first established in 1971, Mr Gillespie will take over from Mr Judd Buchanan, who was promoted in a Cabinet shuffle to president of the Treasury Board.

One of the new Minister's first tasks will be to sort out the confusion created in the minds of many Canadian scientists by recent moves over the funding of research. In particular, a strategy announced earlier this year by Mr Buchanan pledging greater support for research and development has been followed by budget cuts severely reducing various areas of federally-funded research, particularly in government laboratories.

R&D policy has recently become a hot political issue in Canada, figuring prominently in the pre-election campaigns of all the main political parties. This prominence is based largely on the feeling that many of the country's economic problems have been due to its relatively low expenditure on research compared to other industrialised nations.

In June of this year, Mr Buchanan announced a number of funding increases and other measures designed ultimately, he claimed, to raise the proportion of the gross national product spent on R&D from 0.9% to 1.5% by 1983 (the comparable figure for countries such as West Germany and the US is about 2.5%).



Alastair W. Gillespie

In line with this strategy, a pre-election budget presented to the Canadian Parliament last month by Prime Minister Pierre Trudeau included a number of tax incentives to encourage private companies to invest in R&D. In particular, firms taxable at the small business rate will receive a new investment tax credit of 25% on all R&D expenditure; for other firms, the basic rate of the R&D investment tax credit will be doubled from 5 to 10%.

The Government's declared commitment to increase R&D support have

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been welcomed by Canada's Science Council, which has recently been arguing that such moves are necessary to promote the country's technological autonomy. "We are delighted with the new tax incentives, particularly those for small businesses, which tend to be an important source of industrial innovation, and where Canadian ownership is concentrated", the council's executive director, Mr John Shepherd, said last week.

Members of the scientific community, however, while welcoming the Government's commitment, are concerned that too much emphasis may be placed on relatively short-term research objectives, with a corresponding neglect of more long-range, basic research. And widespread concern was expressed in September when it was announced that a number of areas of federally-sponsored research would be cut back as part of a package of austerity measures.

The effect of the cuts, which included closing down a number of internationally recognised government research stations, has been to reduce the 1979/1980 research budgets of the Department of Agriculture by $3.5 million, of Energy by $11.7 million, and of Fisheries by $11.1 million.

Budget increase of $5 million for the Natural Sciences and Engineering Research Council and $3 million for the Medical Research Council, announced by Mr Buchanan in June will remain relatively intact, with only $500,000 being taken away from each. However a $2 million reduction in the budget of the National Health Research and Development programme will mean the termination of several health care research programmes.

The Canadian Association of University Teachers and other university-based organisations have sent a telegram to Mr Trudeau warning him of the "disastrous" implications of the cuts for higher education, research and health care.

Public expressions of concern have not been without their effect. Last month the Government reversed its decision to close one of the oldest research stations in Canada, that attached to an experimental forest at Petawawa, Ontario; following considerable outcry, it has been agreed to keep the research station open, and to amalgamate into it two research groups currently based in Ottawa, namely the Forest Fire Research Institute and the Forestry Management Research Institute.

Nor has a number of universities been slow to realise that the future survival of their research efforts may depend on the extent to which they can build up a working relationship with both government and industry. This week, a two-day conference bringing together leading representatives of the three constituencies to work out mechanisms for co-operation is being held in Saskatoon, an event which some observers feel would have gained little support 10 years ago.

The scientific community must do more than simply complain about reductions in budget expenditures, says Dr John Kucharcyzk of the CFBS, which has organised the conference. "Instead we must continue to stress the importance of a vigorous scientific effort to reach Canada's overall economic objectives."

David Dickson

NASA plans a new Venus mission

As US scientists prepare to analyse the data from the four probes that began their descent through the atmosphere of Venus this week, the National Aeronautics and Space Administration is already working on plans for a follow-up mission to the planet.

The current Pioneer Venus mission is primarily concerned with sending back data on the atmosphere of Venus. In contrast the new mission, which NASA hopes President Carter will include in his budget request to Congress for the fiscal year 1980, would gather detailed information about the planet's surface and its internal structure.

The proposed Venus mission is at present top of NASA's priority list for new projects in the planetary sciences. Close behind is a project, scheduled for possible inclusion in the 1981 budget, to send a spacecraft past Halley's Comet as it approaches the sun in 1985 and on to an encounter with the Comet Tempel 2.

Both missions are expected to cost between $300 and $500 million, and are within the framework of the US Administration's policy for space science announced two months ago. In a "normal" year, NASA officials feel the new Venus mission would stand a high chance of being financed; but with the country in a cost-cutting mood, it remains to be seen whether NASA's request survives the budget process.

The new mission is known as the Venus Orbiting Imaging Radar (VOIR), and would involve a spacecraft circling the planet for at least seven months, providing the first detailed global view of its surface.

In the current mission, Pioneer Venus 1 will orbit the planet, and send back radar pictures. Their resolution, however, will not be much better than that which can already be obtained from Earth. The Soviet Venera missions have already sent back pictures transmitted from the surface of the planet, but only from three fixed locations.

The new spacecraft would, if it receives approval, be launched in December 1984 to arrive at the planet in May 1985. Radar mapping of the surface will be carried out using the synthetic aperture radar (SAR) successfully tested on the ill-fated Seasat mission; this will provide near-global coverage of the planet in moderate resolution, and coverage of a pre-selected small percentage of the planet's surface in high resolution (down to about 50 metres).

In addition to the SAR, the VOIR orbiter will also carry an altimeter and a magnetometer. It is hoped that the spacecraft will disclose the presence or absence of continents, ocean basins, volcanoes and other geophysical phenomena, as well as the nature and time sequence of any plate tectonic activity.

The proposed mission to the Comet Temool 2, which circles the sun once every five years, follows the abandonment last year of more ambitious plans for a close-range study of Halleys Comet, a mission for which NASA reluctantly decided it would have been too expensive to develop an appropriate propulsion system.

Part of the problem is that Halley's Comet circles the sun in an opposite direction from the earth, and a close encounter would have involved elaborate manoeuvring. The new plan is for a spacecraft propelled by the ionisation of mercury vapour to be launched on a course that passes close to the comet, dropping a probe into its tail, and then continues to a position that would allow it to travel alongside Comet Tempel 2 three years later.

Other projects submitted by NASA to the White House for possible inclusion in the 1980 budget include a gamma-ray observatory designed to study, for example, the high energy nuclear and gravitational processes which occur in the vicinity of neutron stars and black holes; an an advanced X-ray atrophysics facility which will provide high resolution data on the position and structure of celestial objects such as pulsars, binary-star systems, and quasars.

David Dickson

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news in brief

● **Birmingham University to be prosecuted:** The UK Health and Safety Executive is to prosecute Birmingham University following the smallpox outbreak from the medical school last August which led to the death of Mrs Janet Parker, a medical photographer, and was followed by the suicide of Professor Henry Bedson, who was head of the laboratory. A date for the start of the hearing has yet to be fixed; a spokesman for the HSE believed it would be early next year.

The university is to be prosecuted for "alleged failure to ensure, so far as was reasonably practicable, the health of its employees in the east wing of the medical school," according to a statement by the HSE last Thursday.

In a statement, the HSE reaffirmed the need for all medical establishments to make sure that the necessary precautions were observed in dealing with all category A pathogens, especially smallpox. The safety guidelines are contained in the HSE publication "Control of laboratory use of pathogens dangerous to humans".

The HSE is to base its case on its own investigations. Meanwhile, Professor Reginald Shooter, chairman of the Dangerous Pathogens Advisory Group, is conducting an official government inquiry into the incident, which is also expected to be completed early next year.

● **US asked to reveal data on A-bomb tests:** The US Government has been asked to declassify all documents on atmospheric tests in the 1950s in an attempt to determine whether the tests were responsible for an abnormally high incidence of cancers in populations exposed to radioactive fall-out. The request has come from Governor Scott Matheson of Utah, who has expressed concern at an 11-year-old unpublished study by health officials which shows unexplained clusters of leukaemia victims in southern Utah in the path of fall-out from atomic bomb tests that took place between 1951 and 1962.

Thirty-eight claims have been filed against the Department of Energy by attorneys for survivors of cancer victims in southern Utah and Northern Arizona seeking more than $1 million in damages for each of nine cancer deaths. It is expected that up to 250 further such claims will be filed in the near future. The claims are the first to have been filed against the government on behalf of civilians not directly connected with the bomb testing programme, but merely living in the path of radioactive clouds.

Last week a federal judge in Newark, New Jersey ruled that the US Army must surrender films and recordings of atomic bomb tests conducted in 1953 in order that a 47-year-old cancer victim, who says he was forced to witness a blast, can compile evidence in a $13 million negligence suit.

● **£350,000 science awards:** Victor Hasselblad (right), inventor of the famous Hasselblad camera, who died earlier this year, left part of his fortune to establish awards similar to the Nobel prizes. From March next year, prizes worth a total of £350,000 are to be awarded each year to selected scientists for research into the natural sciences. The awards will be chosen by the Erna and Victor Hasselblad Foundation. According to a government official in Stockholm, Victor Hasselblad's instructions for the selection of scientists and the areas of the natural sciences for which the awards will be given, are in a sealed envelope which has yet to be opened by the executors of his will.

● **Lerner still cannot leave Russia:** Dr Aleksandr Lerner (left), the Moscow cyberneticist, has taken over the unenviable position as doyen of the *refusnik* scientists, now that Veniamin Levich has finally arrived in Vienna. Lerner, who is 66, has been waiting for an emigration visa for Israel for seven years. In the meantime, he has been working privately on a project for an artificial heart.

According to his daughter Sonia Levin, who visited London last week, Dr Lerner has now completed the basic control programme and the main priority is now for him to be able to collaborate freely with haematologists to establish the reaction of the blood to such an artificial organ. This, however, is not possible in the limbo conditions of a *refusnik* and the Soviet authorities have intimated to Dr Lerner that they would rather see him inactive in Moscow than doing useful work elsewhere.

Furthermore, some kind of personal reprisal seems involved due to Dr Lerner's activity in the Jewish emigration movement. US Congressman Rhodes, leader of the Republican party in the House of Representatives, says he was informed by the Soviet ambassador to the USA, that Dr Lerner would never be granted an emigration visa, because he is a "trouble maker".

● **Lead absorption higher than expected:** Scientists at the Atomic Energy Research Establishment at Harwell have shown that the proportion of lead from car exhausts absorbed by the human body is higher than previously expected. Denis Howell, Minister of State for the Environment, said: "This is an important finding and may be particularly significant where people are exposed to high levels of airborne lead from petrol."

The scientists confirmed that the majority of lead in blood comes from eating and drinking; lead from the air generally contributes less than 10% of the body's total uptake. However, at high concentrations, for example, people living near the busiest stretch of the M4, the contribution of air lead is comparable to that from diet. Sixty per cent of the lead inhaled by volunteers sitting near a motorway was deposited in the lungs. This finding agreed with earlier estimates. But the percentage of lead deposited in the lungs for people near urban roads was much higher than expected, at 48%.

The effect of lead on health, which was not considered by the scientists at Harwell, is to be assessed by a working party of independent experts which has been set up by the Social Service Secretary.

● **Kennedy plans hearings on biomedical research:** Senator Edward Kennedy has announced that the Senate health and science subcommittee of which he is chairman will hold a series of oversight hearings into the funding of biomedical and behavioural research early in the next session of Congress. Speaking during a ceremony to present the Albert Lasker awards for public health service and basic medical care research, Mr Kennedy said that in 1977 the US invested only 3.4% of its "health care dollar" in biomedical and behavioural research, compared with 4.9% in 1967.

"That decline in our overall commitment to research is short sighted and regrettable. It cheats our health care system of potential breakthroughs which will ultimately permit us to restrain health care costs without harsh regulatory controls. It has become apparent that our government has no clear system for setting priorities."

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Risk assessment is more than numbers, Swedish seminar concludes

By Wendy Barnaby

"NEAR Santa Barbara, California, plans have been made to construct a large liquid natural gas terminal . . . A small group of American Indians is protesting the siting of the plant, contending that the chosen location is the most sacred spot of their culture, the Western Gate, where the Indians say the souls of their people must pass after they die to join the spirits of their ancestors . . . One can envision a Rasmussen-like report, examining the probability that a soul would be unable to migrate past a liquid gas terminal. The report would, no doubt, conclude that the risk was very small, perhaps 10^{-8} for each soul. To technical experts the risk seems negligible; to the Indians the risk is unthinkable . . ."*

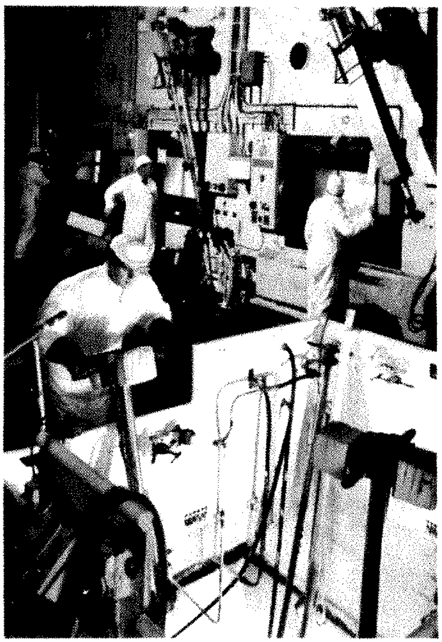
How can risk impact assessment (RIA) deal with value judgements? This emerged as one of the main themes of a three-day long seminar entitled "Impacts and Risks of Energy Strategies: Their Analysis and Role in Management", held recently in Stockholm. The answer, simply stated, is that nobody knows.

Scientists evaluate the risks involved in a proposed course of action; policy-makers draw up policies after they have weighed the risks in a social context; organisations carry out the policies—so the theory goes. The trouble is that this cycle involves scientific, social, psychological, political and managerial processes and disputes, and it is very hard to say how—or even by whom—they can all be brought together in some coherent whole.

According to Dr Baruch Fischhoff from Decision Research in Oregon, USA, RIA is limited by not having some inputs necessary for the analysis, by the analysts' inability to assess the validity of their work and incorporate that assessment into guidelines for action, and for its failure to address critical management issues. Some of the missing inputs are the preferences and values of the people who will be affected by whatever action is proposed: there is no adequate way of incorporating them into the numerical calculations that RIA demands. David Pearce, Professor of Political Economy from the University of Aberdeen, examined the issues that arose in the Windscale inquiry into whether or not to build an oxide fuel reprocessing plant in Cumbria, England, to see what—if anything—RIA could contribute. It is, he concluded, a valuable tool for ordering thoughts, but in considering issues like constraining the liberty of the individual or risking nuclear weapons proliferation and war, it has little or no role to play. RIA is, he inferred, unable to deal with ethical and political choices.

The problem of who should evaluate what in the RIA cycle was nicely illustrated by a difference of opinion between Dr Timothy O'Riordan from the University of East Anglia, and Mr Carl Tham, now Sweden's Minister for Energy. Arguing that the technically-sophisticated identification and estimation phases of RIA have been more fully developed than the evaluative and control phases, Dr O'Riordan said, "Ideally, the basis upon which key decisions are made in the evaluative and control aspects . . . should be made fully public and should be subject both to extra-parliamentary and parliamentary debate." Taking the academic's view, he saw this as a means of incorporating values into the cycle, to counter our present blind faith in scientific advice. Mr Tham, on the other hand, recommended quite a different procedure. "Politicians or decision-makers are best served with hardware facts about risks and their impacts on health and environment", he said. "Then it's our (ie the politicians') job to evaluate these facts as sensibly as possible and to make the necessary appreciation of costs and benefits." But can the politicians assume that they understand how the public perceives issues? There is "an immense gap between the views of technical experts and the views of a significant portion of the public", concluded Drs Paul Slovic, Sarah Lichtenstein and Baruch Fischhoff of Decision Research after studying opponents of a nuclear reactor to see how they perceived the threats it posed. Are the politicians informed and impartial enough to make their policies reflect these perceptions?

The RIA cycle does not end with the policy-makers, either: management, generally in the form of organisations, then takes over. But there is no guarantee that what is actually done will be even vaguely like what the policy-makers intended to have done: a gap demonstrated by Dr Shelagh Staynes fom London University's Monitoring and Assessment Research Centre, who talked about the administration of lead pollution ocntrol in the UK.



Reprocessing at Windscale: the risk may be high or low, but how to deal with ethical and political choices?

A more surprising difficulty with RIA is that there is so little communication between the different groups involved in it. The seminar—held jointly by the International Institute for Energy and Human Ecology (the Beijer Institute) and the Swedish Energy Research and Development Commission in association with UNEP—did at least get these groups talking to each other. Pointing out that natural scientists, politicians, psychologists and ecologists all contributed to the meeting, Professor Gordon Goodman, the Institute's Director, said, "There have been few occassions—it happens almost never—when people quantifying objective risk have talked to people trying to measure a democratic society's evaluation of risk perception. The policy-maker relates to the scientist and sometimes to the sociologist, but there has been no logical attempt to bring it all together." As far as Professor Goodman was concerned, however, the seminar summarised the state of RIA art and pointed to areas where more work could usefully be done to try to shed some light on the way many social policies are made. One of these, which the Institute hopes to take up, concerns threats of small probability but high significance: how could these be incorporated into RIA? How can the management process deal with them? Another area is "safety engineering": using RIA to identify technological systems' weak spots and cost-effective methods of improving them. Then there is a need for *post hoc* and comparative risk studies. The list is long, and illustrates how little we know about working out the greatest good for the greatest number. ☐

**From a paper presented at the seminar by Dr Raphael Kasper of the USA's National Academy of Science's Environmental Studies Board.*

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correspondence

Rothschild: an antidote to panic

SIR,—There are a number of inaccuracies or misrepresentations, doubtless unintentional, in the leading article (30.11 78) on my Dimbleby Lecture. I shall give a few examples.

- "Lord Rothschild . . . argued that we should develop a table of risks so that we could compare, say, the risk of our dying in an automobile accident with the risk of Baader-Meinhoff guerillas taking over (sic) the nuclear reactor next door." I specifically said that the risk of such a terrorist gang gaining entry to a nuclear power station was secret and *therefore unknown*
- The leader claims I implied that viewers could not convert "probabilities from one form to another". The reverse is the case as evidenced by the visual example given, actually on a pocket calculator which was seen by the audience. The example was 109÷109=1; 1,000.000—109=9,200. (Must I say, in *Nature*, that 1,000,000÷109=9,174.3119?)
- I was said not to have quoted "a single error on the risks I enumerated". The reason was quite simple the BBC did not like references to confidence limits or intervals, although I spent some time in the lecture on the importance of what I call "tolerances". In addition, I said that "1 in 100 is not exactly 1 in 100 in the world of risks, but, for example, *probably* somewhere between 1 in 95 and 1 in 105" If you had bothered to look at the printed version of the Dimbleby Lecture which was obtainable on 24 November, the day after the lecture was broadcast, you would have found references to confidence limits under six of the seven tables in the lecture. Ranges were given in the other one, Table 6 There are other misrepresentations which I did not expect in an editorial in *Nature*

What is more important is that your leader-writer has failed to understand the *constraints* imposed by talking about a highly complex and technical subject to 5 million people, the overwhelming majority of whom are laymen, in controdistinction to the few thousand who read *Nature*, and the *restraints* imposed by the BBC with their undoubted expertise.

I fear I am not competent to give a lecture on "the democracy of risk assessment", whatever you mean by that phrase. Let us hope your leader writer will soon give it, if only so that we know what it is.

As stated in the last but one paragraph of the lecture (p. 19), two of its main objects were to induce people to think about risks "for a minute or two", and "to compare the different risks around us." If I may quote myself in conclusion (p. 3) "Comparisons, far from being odious, are the best antidote to panic" In the editor's case the panic appears to be about the use of numbers.

ROTHSCHILD

N M Rothschild & Sons Ltd,
New Court, St Swithin's Lane, EC4

SIR,—In your forceful editorial, you assert that the quantitative assessment of risks is not enough and that emotional reactions also deserve a role in decision-making True, and indeed we live in a society where emotion is hardly likely to be under-represented in political decision-making. But emotional reactions are not necessarily and automatically decisive, and surely Lord Rothschild was merely concerned to get across the fact that such a thing as risk calculation exists and that *it* deserves a part in decision-making too Many non-scientists don't know how risks are calculated, especially on an a priori basis; is it arrogant to draw attention to the fact that such calculations *can* be made?

One of the most intractable problems in the discussion of nuclear power (and genetic experiments, et hoc genus omne) is that the expert is the victim of a classic catch-22: he can only acquire expertise of certain kinds, it is implied, by working in the industry or science that is under attack, and that, it is asserted, automatically destroys his objectivity (and the non-objective cannot be a real expert). But if emotional bias is to be automatically presumed in the expert, then how is the undoubted emotional bias of the external attackers (ex hypothesi, they cannot be experts!) to be assessed? Does not that bias nullify the validity of the attack? In this connection it should be remembered that a recent public survey in Britain elicited the fact that a large majority of the public prefers to trust the technical judgment of the experts on nuclear power, intellectual prostitutes though they apparently are

Emotional reaction to such things as nuclear reactors is a slippery basis for decision-making. I don't much like paying taxes, but I would hesitate to put that consideration too far ahead of dull calculations about the need to pay old age pensions somehow. How is allowance to be made for the fact that fears can be whipped up for reasons which quite often are largely political? Is it to be accepted that certain forms of risk are psychologically intolerable however small they are, for inscrutable reasons, whereas others are cheerfully accepted even though not negligible? For instance, it is notable that there are no public attacks on the transport of liquefied natural gas. Rothschild quoted figures implying that this process is even safer than nuclear power, yet a large LNG carrier bears explosive power equivalent to an atomic bomb. Though the LNG disaster involving a lorry in Spain indicates the destructive power of the fuel, the fact that no ship has blown up yet should not (on the analogy of the arguments applied to nuclear power) assuage the fears of those who live in harbour-towns where such ships are unloaded. Why not? Could it be that such an attack would be politically embarrassing beceause most LNG comes from the Third World?

Even if, for argument's sake, one postulates that no one working in the nuclear energy industry is to be trusted to give an honest opinion on contentious matters, it remains true that there exists a distinct group of experts, such as nuclear inspectors and medical radiation specialists, who can cast a cool and independent eye over some of the issues in dispute For instance, Sir Edward Pochin, who is a medical professor, recently gave a lecture entitled "Why be Quantitative about Radiation Risk Estimates?" (National Council on Radiation Protection and Measurements, Washington, D.C , Lauriston Taylor Lecture No. 2, 1978). He makes a number of points in the special field of medical radiation protection which are very reminiscent of Lord Rothschild's and in the last part of his lecture he puts together some risk calculations from the UN, OECD, the International Commission on Radiological Protection, the US National Safety Council and the like (all, one would think, immaculately independent bodies) and concludes that the death risk from the total radiation exposure, from the entire uranium cycle, for an average citizen (including 'nuclear workers' in the average), in a country where one kilowatt electrical power per citizen comes from nuclear stations, is equivalent to the death risk from smoking one cigarette every two years. He adds that he recognises that relative acceptabilities of the two risks is another matter, but believes that "the numerical comparisons of this type do have a certain value in letting radiation be seen in a proper context as one of the numerous potentially harmful components of the occupational, as of the general, environment. . " The essential answer to your objection to Lord Rothschild's lecture, surely, is that it can only be beneficial to have context.

Two other brief points should be made about your editorial. Firstly, you point out the special difficulty of assessing 'unknown risks'—risks of something that has never yet happened, like a serious reactor accident, or a major LNG ship disaster But surely it is a very odd attitude to treat, by implication, something so safe that it has never yet gone seriously wrong over a period of years, as more suspect (merely because no *historical* risk estimate is feasible) than, say, coal-mining or deep-sea fishing? This implies that the more successful an industry is in minimising fatalities (and the nuclear power industry has, to date, a unique record), the larger the part that emotion (as distinct from facts) should play in assessing its desirability. Odd!

Finally: you make the intrinsically very powerful point that one cannot quantify the risk of the loss of some civil liberties if nuclear power expands, or, for that matter, quantify the emotional reaction to that eventuality. But who shall assess the risk of major social disorder occasioned by very serious energy shortages in future if nuclear power is stopped, and what numerical value would such a disamenity carry?

That kind of argument always pulls in two directions.

Emotion is unavoidable in this kind of debate, and your readers will no doubt discern a smidgin of it in this letter. But in science, emotion should be the engine and reason should hold the steering-wheel and press the accelerator and brake. Risk analysis quite properly helps to guide the foot between those rival pedals.

Robert Cahn

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We did not wish to imply that emotional commitment characterised only one side of the nuclear debate—far from it—only that it was impossible to assess risks without taking such factors seriously. Further, the unknown risk referred to was the risk of failure of a commercial scale fast breeder reactor, for which there is no experience.—Ed.

Solar activity and influenza

Sir,—I would like to elaborate further on the remarkable correlation between influenza A viral antigenic shifts and peaks of the sunspot cycle as noted by R. E. Hope-Simpson (*Nature* **275**, 86; 1978).

Ancient "cosmic and telluric theories" regarding influenza epidemics date as far back as the time of Hippocrates (Thomson, D. & Thomson, R. *Ann. Pickett-Thomson Res. Lab.* **9**, 257–261; 1933 and **10**, 1125–1140; 1934). Over half a century ago, F. G. Crookshank (*Mil. Surg.* **59**, 284–290; 1926) defended the hypothesis that influenza pandemics and solar activity were correlated, and this possible relationship was further studied by M. Mygge (*Acta Med. Scand.* suppl. XXXII, 105–134; 1930). Since the discovery of a filtrable virus as the aetiological agent of influenza in the 1930s, the notion of a solar-influenza association has been largely dismissed and forgotten.

Except for the pandemic of 1889, the beginning dates of historical influenza pandemics in the 18th and 19th centuries (Mote, J. R. In: *Viral and Rickettsial Diseases*, Harvard University Press, 257–261, 1933), as well as pandemics and viral antigenic shifts in this century (Kilbourne, E. D. *J. Amer. Med. Assoc.* **237**, 1225–1228; 1977), appear to have occurred in years of high sunspot number. From our present knowledge of influenza, it is not unreasonable to assume that these severe historical pandemics spreading rapidly to world-wide proportions were associated with major shifts in the influenza A haemagglutinin (H) and neuraminidase (N) surface antigens.

The mechanisms on how the solar cycle can affect influenza pandemics may only be speculated upon at this time. The amount of ultraviolet light incident on the surface of the earth is increased in years of high sunspot activity (Pettit, P. *Astrophys. J.* **75**, 185–221; 1932), and this can conceivably accelerate the mutation rate of the influenza virus. The relationship between long-term cyclical fluctuations in meteorological and climatic conditions with the sunspot cycle remains controversial at this time. It is plausible, nevertheless, that during years of high solar activity, a more favourable ecological environment may exist for the genetic recombination of human and animal influenza viruses, with the emergence of new pandemic strains.

It is interesting to note that while the swine influenza virus (HswlNl) reappeared in 1976, its spread into pandemic or even epidemic proportions did not occur in this year of sunspot minimum. The resurfacing of the HlNl subtype ("Russian flu") in December of 1977 was completely unexpected. The sunspot cycle was beginning its rapid ascent at that time.

It would not at all be surprising if a new strain of influenza A virus should emerge and dominate in the sunspot maximum years of the near future. On the other hand, perhaps what could only be expected from the elusive influenza virus is the unexpected.

Roy Ing

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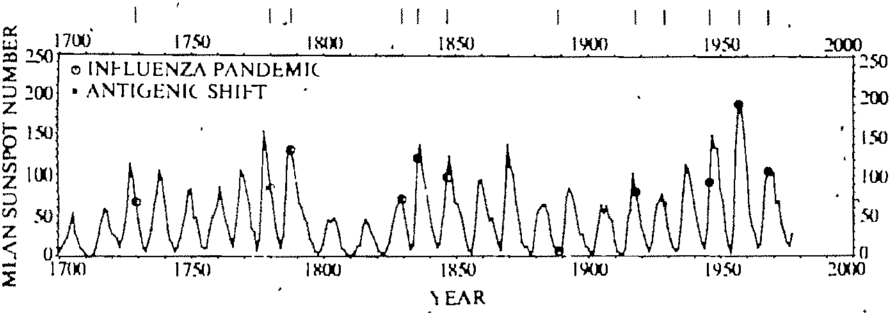

Seveso: premature optimism

Sir,—At the recent Seventh International Congress of Pharmacology in Paris, Dr G. Reggiani of the Hoffman-La Roche Medical Board presented a communication on the clinical features of the July 1976 TCDD accident in Seveso. The Mario Negri Institute have closely followed many aspects of the investigations since then, and we feel some comment is necessary.

TCDD (2,3,7,8 tetrachlorodibenzo-p-dioxine) is one of the most powerful known toxic chemicals in animals, especially as regards its cumulative and delayed effects. So there cannot but be serious concern about the long term effects of the accident on man, regardless of the present impossibility of making any defensible prognosis (S. Garattini *Biomedicine* **26**, 28–29; 1977).

Since the accident, Hoffman-La Roche, which owns ICMESA through its subsidiary Givoudan, has been faced with many problems, of which public relations is certainly not the least. Its representatives have with every right denied false or exaggerated press reports such as charges that ICMESA had been secretly engaged in manufacturing warfare chemicals. After a while, though, they began to comment optimistically on the potential health risks to people living in the polluted area. For example, Dr Reggiani noted that: "In the case of TCDD man seems to respond to a certain extent differently from other animal species . . . " (J. Walsh *Science* **197**, 1064–1067; 1977). There is no question that all efforts to minimise exposure of the residents in the disaster area should not be discontinued and we fervently hope Dr Reggiani's opinion proves right. But he seems to be rushing it somewhat by extending his increasingly sanguine attitude to retrospective evaluation.

His Paris communication was obviously biased by his desire to make little of the possible damage to human health. Thus he offered reassuring interpretations of findings such as those on chloracne, the typical skin lesion after TCDD exposure in humans, pointing out its "quick healing tendency" with the unsupported implication that it was quantitatively linked to exposure. He failed to mention its recurrence in people from widely scattered areas. Regardless of whether they were based on poorly reliable data such as observed rates of spontaneous abortion, Dr Reggiani confidently presented negative findings as strong evidence for the absence of TCDD induced pathology, with no criticism of the epidemiological methodology adopted or related problems deriving from the lack of adequate historical controls.

These points are evident from his abstract in the official volume (*Abstract n.* 2890, 953) which concludes: "The measures of protection of the population and prevention of further damage carried out at our request by the local authorities have avoided the severe injuries observed in previous similar accidents".

In view of this statement we must recall that the Seveso case cannot be compared with any previous accident involving TCDD because of 1) the uprecedently heavy contamination of inhabited areas, 2) the delay in recognising the nature of the accident and the resulting lag before protective measures were adopted, and 3) the significant amounts of TCDD persisting in the environment where people live. No mention of the TCDD risk was made to the local authorities before the disaster and even after it. Roche took almost two weeks to admit that TCDD had escaped from the plant.

It is unfortunate when someone takes advantage of a scientific meeting to present such misleading statements. People with no knowledge of the background and complexity of the Seveso case and the difficulty of epidemiological studies, may be led to accept the idea that thanks to Roche, all is well in Seveso now. This sounds outrageous to those who have already suffered distress from the disaster, apart from all considerations of feared future effects on health, which only time will reveal.

In addition and perhaps most relevant, our concern about Dr Reggiani's statement is of practical value. Everybody knows how difficult it is to motivate people to keep coming back for the controls needed for epidemiological studies. If the idea is spread around that there are no risks it will be difficult to collect long term data. No data available may well represent the best possible proof that no toxic effects occurred!

L. Manara, S. Garattini

Istituto di Ricerche Farmacologiche
"Mario Negri", Milan, Italy

Reflections on the occasion of my birthday, by Mr Davy

It has been a matter of some gratification to me, in my present rather distant circumstances, to note that the Anniversary of my birth has not gone unnoticed by your esteemed Journal. I refer, Sir, to the remarkably accurate and well executed likeness of the lamp which is associated with my name, that occupies the entirety of your frontispiece. By the yardstick of eternity two hundred years is a mere nothing It would be churlish to complain of a lack of recognition, were it not that various dabblers and meddlers have received more than their proportionate share of such credit as your transitory World offers. It is true that the Royal Institution has seen fit to mark this Anniversary with an exhibition. But why is access restricted to Tuesdays and Thursdays between the paltry hours of noon and four? And why do they charge a derisory fleabite of only twenty pence? In 1811, in Dublin, tickets cost two whole guineas to hear me lecture on 'Peculiar Observations in Electrochemistry' And even so all five hundred and fifty tickets were sold within a week. Subsequently I was told the self-same tickets changed hands on the street at up to twenty guineas apiece! In those days Chemistry was much more popular as a subject for lectures, than Astronomy, Mechanics, or Natural History. A critic wrote, 'These subjects are not susceptible of any brilliant exhibitions, there is no noise, no fire—and the amphitheatre never fills, but for Mr Davy. A small bit of Potassium thrown in a glass of water, or upon a piece of ice, never fails to excite a gentle murmur of applause! And the audience consisted of young Gentlemen together with an equivalent number of young Ladies of High Quality.'

I know that Dalton and Faraday have done very well since—they have Divisions named after them in the Chemical Society. But as for Dalton all that talk about atoms; it was mere speculation, no experimental evidence As I said at the time, 'it's more ingenious than important'. And as for Mr M. Faraday, I very much resent those who say that he was my greatest discovery. Let me remind you that, whatever became of him afterwards, he was only my Assistant. He may have discovered the Laws, but I discovered the Elements: Sodium, Potassium, Calcium, Magnesium, Barium, Strontium and Chlorine Electricity from my Giant Pile was able to exert a power on substances such as had not been exerted before. Even mighty Soda and Potash yielded themselves to sustained attack. Not all Savants could accept my simple view of the little molten globules emerging through the crust of the potash and bursting into purple flame. This was not to be the last time that Gay-Lussac and his henchman Thenard obstructed the course of Natural Philosophy. Carried away by their isolation of Boron—a remarkable achievement for them—I may have perchance referred to Gay-Lussac as being the Head of Living Chemists in *France*. But their notion that *my* Potassium was a mere Hydride of Potash could not be sustained and can only be regarded as being vexatious. No wonder I resent Gay-Lussac's Bicentennial—200 francs a head, no half price for children and open all the week including Sundays! I grant you that M. Lussac was a competent experimentalist; somehow his numbers always fitted. But Laws are merely reflections of Man's puny attempts to describe the omnipotence of the Supreme Being:

> "All things most glorious on the Earth
> Tho' transient and short lived they seem,
> Have yet a source of heavenly birth
> Immortal,—not a fleeting dream"

And I speak of Experiments, not Laws As I once said, 'It is more laborious to accumulate facts than to reason concerning them; but one good experiment is of more value than the ingenuity of a brain like Newton's.'.

My first experiments were conducted in Mr Tonkin's house in Penzance and relied on such apparatus as wine-glasses, tea-cups, tobacco-pipes and crucibles, securing preference upon the kitchen stove to the more ordinary domestic comestibles. I shall always be beholden to the generosity of Dr Thomas Beddoes and the Pneumatic Institute of Bristol but it was at the Royal Institution, following the work of Nicholson and Carlisle, that I demonstrated that Hydrogen and Oxygen alone were the true products of galvanic action on pure water. I discovered to my manifest astonishment that the two gases could be separated if two gold plates, dipped into separate vessels of water were linked by the fingers of my own hands This was not the first time that my body had been used as a tool in the Laboratory And, a fig, Sir, for your Health and Safety at Work Act! In 1806 I demonstrated the relation of electricity to chemistry using three vessels connected by moist fibres In front of the very eyes of an audience, I immersed metal plates in two outer vessels containing Barium Nitrate and Sodium Sulphate and these outer vessels were linked each to the other through a third vessel containing pure water. The action of the Giant Pile on this apparatus brought forth a magnificent deposition of solid white Barium Sulphate in the middle vessel. Such a spectacle could only be interpreted as a manifestation of Nature's innermost Secrets. For the first time I realised that some substances were attracted and some repelled by the quality of the electrification of the plates. I declared 'These attractive and repulsive forces are sufficiently energetic to destroy or suspend the usual operation of Chemical Affinity'. One cannot speculate with profit on the nature of electricity, but I had no doubt it was connected with Chemical Affinity and the very essence of Matter.

The Lamp you have, Sir, in your frontispiece, should not be the only reminder to your Readers of the festive Season. Why, Sir, on Boxing Day—aptly named—in 1799 I discovered the medical properties of Nitrous Oxide. I enclosed myself in an airtight breathing box, having a cubic capacity of about nine and a half cubic feet. I was in the box for one and a quarter hours, and breathed over twenty quarts of pure nitrous oxide. A thrilling extending from the chest to the extremities was almost immediately produced. I felt a sense of tangible extension, highly pleasurable in every limb; my visible impressions were dazzling and apparently magnified. By degrees as the pleasurable sensations increased, I lost all connection with external things; trains of vivid visible images rapidly passed through my mind and were connected with words in such a manner, as to produce perceptions perfectly novel. I existed in a World of newly connected and newly modified ideas I theorised; I imagined that I made discoveries. And that is why, Sir, I crave the courtesy of your columns to enquire why I should be remembered only by my Lamp (as if I was Miss Nightingale) rather than as the Father of British Anaesthesia. I need hardly remind you that the Poet Southey wrote 'Davy has invented a new pleasure for which language has no name. I am going for more this evening, it makes me strong and happy! So gloriously happy! Oh, excellent air-bag!' And in 1801 Wordsworth and Coleridge beseeched me to teach them Chemistry in a special Laboratory to be constructed at Keswick. Had I acceded to their request and gone to the Lake District, rather than the Royal Institution, the benefits to English Literature and to Chemistry remain to this day, Sir, incalculable

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John Albery and Peter Barratt, of Imperial College, claim to have found this letter.

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news and views

Intervening sequences in the mitochondrial genome

from Giorgio Bernardi

SINCE its foundation by Boris Ephrussi 30 years ago, mitochondrial genetics has made great advances and yet this subject has remained an area of interest to only a small number of laboratories. Whatever the explanation for this, the situation is not likely to last much longer since exciting results recently obtained in one particular system, the mitochondrial genome of yeast, should stimulate very wide interest.

The mitochondrial genome of *Saccharomyces cerevisiae* comprises about 100 identical, circular, supercoiled DNA molecules per diploid cell. Each genome unit is about 50 million in molecular weight, a value five times greater than that of the mitochondrial genome of organisms as far apart in terms of evolution as *Chlamydomonas* and man, but smaller (by a factor of almost two) than that of higher plants. Each genome unit is known to encode two ribosomal RNAs, at least 30 tRNAs, and nine proteins: three of the seven subunits of cytochrome *c* oxidase, one of the seven subunits of the *bc1* complex, three of the ten subunits of the ATPase complex, and one protein, *var1*, of unknown function. The other mitochondrial proteins are specified by nuclear genes, a fact which emphasises the very close cooperation between nuclear and mitochondrial genes in mitochondrial biogenesis and function.

The fivefold difference in unit size between the mitochondrial genomes of yeast and, say, man, is difficult to explain in terms of coding potential since it is unlikely that the former codes for so many more gene products than the latter. Put in a different way, the discrepancy between the unit size of the mitochondrial genome of yeast, about 50 million, and the amount of DNA required to encode its known gene products, about 10 million, raises the same problem as the so-called 'c-value paradox' of the nuclear genome of eukaryotes, namely, what is the role of the 'excess DNA'? An answer to this problem is emerging from two different experimental approaches

A direct molecular approach revealed, some years ago, that half of the mitochondrial genome of yeast consists of AT spacers (GC<5%) formed by short, repeated dAT : dAT and dA : dT sequences. The other half, presumed to correspond to the structural genes and to their regulatory elements, is extremely heterogeneous in base composition (since it ranges from 21% GC for ribosomal genes, to about 35% GC for tRNA genes, to 65% GC for GC-rich segments representing 10% of the genome) and contains 60–70 clusters of the sequences CCGG and GGCC[1] which are palindromic and homologous in sequence[2,3], as originally postulated by Prunell and Bernardi. By cutting these clusters with the restriction enzyme *Hae*III, the genome can be split into 60–70 fragments, most of which seem to be formed by a GC-rich region, a gene (or gene segment, see below), and a spacer. Interestingly enough, the *Hae*III fragments vary in length and (to some extent) in number in different strains, two factors which are responsible for a variation of ±5 in the size of the corresponding genomes[4].

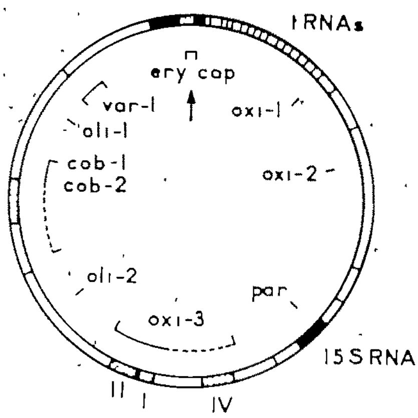

Fig. 1 The mitochondrial genome of *S. cerevisiae* modified from Borst & Grivell *Cell* (in the press). The location of the genes for 21S and 15S RNAs is indicated. Thin, radial lines indicate the position of tRNA genes. Roman numerals indicate genome segments absent in *S. carlsbergensis*. The 21S RNA insert is represented by the dotted region splitting the 21S RNA gene. The approximate location of a number of genetic markers as well as the *cob* loci referred to in the text is indicated inside the ring. The arrow indicates the origin of the map which corresponds to a *Sal*I site.

The functions of the spacers, of the (CCGG, GGCC) clusters and of the GC-rich segments are still a matter of conjecture. The presence in them of homologous or quasi-homologous sequences appears, however, to be responsible for the high frequency of recombination phenomena which characterise the mitochondrial genome of yeast. Unequal crossing-over events seem to account for the changes in length of *Hae*III fragments, which accompany genome evolution[4] and genome recombination in sexual crosses[5] as well as for the excision of genome segments leading to the defective genomes found in spontaneous 'petite' mutants[6].

Giorgio Bernardi is Head of the Laboratoire de Génétique Moléculaire, Institut de Recherche en Biologie Moléculaire, Paris, and Co-Director of the Institute.

1. Prunell & Bernardi *J. molec. Biol.* **86**, 825 (1974), **110**, 53 (1977).
2. Macino & Tzagoloff *Proc natn Acad. Sci U.S.A* (in the press)
3. Cosson & Tzagoloff submitted to *J biol Chem*
4. Prunell *et al* *J. molec Biol.* **110**, 17 (1977)
5. Fonty *et al.* *J molec Biol* **119**, 213 (1978)
6. Fonty *et al* submitted to *J molec Biol*
7. Tzagoloff *et al.* *J Bact* **122**, 826 (1975)
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9. Borst & Grivell *Cell* (in the press)
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11. Faye *et al* *Molec. gen Genet.* (in the press).
12. Hahn *et al* submitted to *Cell*
13. de Vries & Kroon *Neurospora Newsletter* No 25
14. Sanders *et al* *Molec gen. Genet* **157**, 239 (1977).

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The starting point for the genetic approach was the discovery of the so-called mit$^-$ mutants in which mitochondrial functions are impaired by point mutations or small deletions affecting the mitochondrial genes coding for the subunits of respiratory enzymes or of ATPase. Mapping work in several laboratories has revealed that both the *cob* (or *box*) region, coding for apocytochrome *b* (the mitochondrial subunit of the *bc1* complex), and the *oxi-3* locus (which codes for subunit I of cytochrome *c* oxidase) correspond to DNA segments several times larger than expected (see Fig. 1). More recent work[8] indicates that non-coding regions and regulatory sequences are present within the *cob* region. The data of Slonimski *et al.*, for instance, indicate that mutants lacking functional cytochrome *b* map in seven discrete clusters dispersed over 6,000–10,000 base pairs. Mutations at non-adjacent loci 4/5, 1 and 6 fall into a single complementation group and produce abnormal apocytochrome *b*. On the other hand, mutations at loci 2, 3 and 7 complement mutations at loci 4/5, 1 and 6, complement each other, and present a disturbed synthesis of both subunit I of cytochrome *c* oxidase and of cytochrome *b*. Most of these pleiotropic mutants are temperature-sensitive and accumulate multiple 'new' mitochondrial translation products. The genetic and biochemical work just mentioned is further supported by electron microscopic work[9] on hybrids between the 18S mRNA transcribed from the *cob* region and restriction fragments from the same region; this shows that the gene for cytochrome *b* consists of at least five coding regions separated by four non-coding regions which are between 600 and 2,000 base pairs long.

At least one other mitochondrial gene, the gene for the 21S RNA, is also split, in addition to the two already mentioned. Electron microscopy of 21S RNA with a restriction fragment containing this gene, show that this contains a 1000 base pair insertion which is closely correlated with the ω^+ allele, a polarity locus affecting the transmission of neighbouring genetic markers in crosses[10,11]. Strains having the ω^- allele lack the insertion, which is always transmitted in $\omega^+ \times \omega^-$ crosses to the ω^+ progeny. Very interestingly, a large intervening sequence[12] containing tRNA genes[13,14] has been found in the gene for the 24S RNA of *Neurospora* mitochondria.

An important question raised by these findings concerns the relationship of intervening sequences with the sequence elements (spacers and GC-rich segments) discussed above. More specifically, it may be wondered whether some of the homologous sequences present in those elements are not used in the RNA splicing. While current biochemical and genetic work will certainly lead to an answer to this question, it is noteworthy that the large regions of strain-dependent genome variability are correlated with the presence of long dA:dT sequences and map at the *cob* and *oxi-3* regions (Fig. 1). Finally, it should be noted that while the presence of split genes further weakens the hypothesis of the prokaryotic origin of mitochondria, its main interest undoubtedly resides in the fact that intervening sequences have now an easy genetic handle which should allow us to gain a better understanding of these puzzling genome elements. ☐

Enzyme defects and immune dysfunction

from Fred S. Rosen

GENETIC deficiency of two enzymes in the purine degradation pathway, adenosine deaminase (ADA) or nucleoside phosphorylase (NP), results in fatal immunodeficiency disease. E. Giblett (University of Washington, Seattle) discovered ADA deficiency serendipitously while examining erythrocytes for informative enzyme polymorphisms from a child with severe combined immunodeficiency (SCID), a profound deficiency of T and B lymphocytes. She subsequently discovered NP deficiency in a child with severe T cell deficiency. These are the only genetically determined immunodeficiencies that have a known biochemical basis. Although ADA and NP are distributed in all mammalian tissues, only the lymphoid system is affected by the deficiencies.

A meeting was held recently at the Ciba Foundation* to discuss the biochemical consequences of ADA and NP deficiency, particularly as to how they affect the ontogeny of lymphocytes.

Both ADA and NP deficiency are inherited as autosomal recessive phenomena. ADA has been assigned to chromosome 20 and NP to chromosome 14. ADA deficiency results in a more profound immunodeficiency than does NP deficiency. Of all infants affected with the autosomal recessive form of SCID, 35 to 50% have ADA deficiency. NP deficiency is known in only six kindred. Affected children in these six kindred have a severe A cell deficiency, normal immunoglobulins, increased antibody responses, autoantibodies, and poor *in vitro* responses to mitogenic stimuli (A. Ammann, University of California, San Francisco). By contrast, ADA deficiencies have a profound defect of both T and B cells and no *in vitro* or *in vivo* immunological function. Although the erythrocytes are completely deficient in ADA, a mutant enzyme has been found in fibroblasts and lymphocytes in low amounts (approximately 15% of normal). This has enabled the prenatal diagnosis of this disease in amnion fibroblasts (R. Hirschhorn, New York University, New York).

It was found that NP deficients accumulate 2′-deoxyguanosine triphosphate (dGTP) in erythrocytes, and lymphocytes whereas ADA deficients have abnormally high levels of 2′-deoxyadenosine triphosphate (dATP) in these cells (A. Cohen & D. W. Martin, University of California, San Francisco). D. A. Carson (Scripps, La Jolla) showed that preferential T cell trapping of deoxyadenosine and deoxyguanosine and subsequent accumulation of dATP and dGTP is due to the high kinase content of T cell lines and their relative poverty of 5′-nucleotidase. L. Thelander (Karolinska Institute, Stockholm) obtained calf thymus ribonucleotide reductase in high purity. The allosteric regulation of this enzyme requires 1×10^{-3} M ATP as a positive effector, and is readily inhibited by dATP and to a lesser extent by dGTP and dTTP. dCTP has no inhibitory effect. In fact, dCDP can rescue murine mutant S49 lymphoma cells that have ADA and/or NP deficiency. N. M. Kredich (Duke University, Durham) offered a very reasonable explanation for the greater severity of ADA deficiency in that adenosine accumulation inhibits the hydrolase of S-adenosylhomocysteine. This substrate accumulates and clogs the methyl donor activity from S-adenosylmethionine. Thus DNA synthesis in ADA$^-$ lymphocytes is obstructed at the ribonucleotide reductase step and DNA methylation is inadequate.

Establishment of lymphoid chimaerism with transplants of histoidentical marrow has been the most satisfactory therapy for ADA deficiency. Lacking a bone marrow donor for an ADA deficient infant, S. H. Polmar (Case Western Reserve, Cleveland) used frozen human erythrocyte transfusions as a source of exogenous ADA. This succeeded in reducing dATP levels in lymphocytes and inducing normal immune function. This procedure was extended with further success to an NP deficient child (B. J. M. Zegers, Wilhemina Kinderziekenhuis, Utrecht). W. N. Kelley (University of Michigan, Ann Arbor) has obtained both ADA

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*Enzyme Defects and Immunity Dysfunction, held 7–9 November. The Proceedings of the conference and the discussion will be published in their entirety for the Ciba Foundation by Excerpta Medica, Amsterdam.

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and tissue ADA binding factor in a high state of purity. Purified ADA awaits trials of its clinical usefulness. Also, on theoretical grounds, deoxycytidine may have therapeutic benefits for NP and ADA deficients.

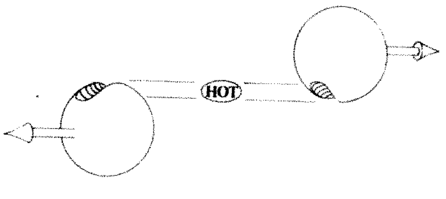
J. F. Smyth (Royal Marsden Hospital, Sutton, Surrey) has infused terminal cancer patients and patients with T cell acute lymphocytic leukaemia with deoxycoformycin, a potent ADA inhibitor. He achieved striking lymphocytocidal effects. Potent immunosuppressive and oncolytic agents may emerge from the study of these rare immunodeficiencies which result from a failure of purine breakdown. ☐

Multipion production in relativistic heavy-ion collisions

J. M. Irvine

IN recent years the nuclear physics community has been excited by the possibility of new accelerator systems capable of producing relativistic heavy-ion beams. There has been theoretical speculation for several years about using such beams for producing new, exotic nuclear phenomena such as superheavy elements, nuclear shock waves, pion condensates (the nuclear analogue of a ferromagnetic phase transition) and quark matter.

If one fires high energy nucleons at one another the most obvious products are pions, and one of the first things to be understood about relativistic heavy-ion collisions then is the pion production. Earlier this year (Fung *et al.* *Phys. Rev. Lett.* **40,** 292; 1978) data became available from the Bevalac at Berkeley on negative pion production in relativistic heavy-ion collisions from beams of ^{40}Ar at 0.4, 0.9 and 1.8 GeV/nucleon and ^{12}C beams at 0.4 and 2.1 GeV/nucleon on targets of LiH, NaF, BaI$_2$ and Pb$_3$O$_4$. As was to be expected theoretical papers aiming to interpret the data have followed thick and fast. The simplest picture (Vary, *Phys. Lett.* **40,** 295; 1978) considers that the colliding nuclei are moving so much faster than the internal velocities of the nucleons of which they are composed that this internal motion can be ignored and the heavy ion collision can be viewed simply as a large number of independent nucleon-nucleon collisions. Vary's calculations predict too many events with high negative pion multiplicities, that is, events where more than 15 negative pions are produced, and attempts to correct this feature by allowing the pions to interact with the nucleons to produce baryon resonances have not been successful. A better account of the pion multiplicities is provided by the fireball model (Gyulassy & Kauffman *Phys. Rev. Lett.* **40,** 298; 1978). In this model, as the target and projectile sweep by each other, the material in the geometrical overlap is swept out and assumed to be at rest in the centre of mass frame. The enormous initial kinetic energy is assumed to be converted into internal thermal energy. The pions are assumed to be produced in this extremely hot fireball fragment (see Fig. 1). Gyulassy and Kauffman conclude that this model is adequate to explain the pion multiplicity data and come to the conclusion that the independent nucleon-nucleon model predicts too many high multiplicity events because it ignores pion absorption. They go on to assert that there is a very large class of dynamical models which will give results identical to those of the fireball model: the only requirements on the models being that the pions are uncorrelated and that the average pion multiplicity is correctly given.



This assertion seems to be supported by recent results of Afek *et al.* (*Phys. Rev. Lett.* **41,** 849; 1978) using the collective tube model. This model uses a geometrical picture of the heavy ion collision like the fireball model and considers the collision of two 'tubes' of nucleons in the target and projectile nuclei. In the centre of mass frame the colliding tubes are Lorentz contracted into narrow disks and the model then assumes that the tube–tube collisions resemble elementary particle–particle collisions. The agreement between the predictions of this model and the data of Fung *et al.* is extremely impressive. Afek *et al.* go on to claim that the agreement between the predictions of the colliding tube model and statistical thermodynamic models, like the fireball model, is entirely accidental and will disappear at higher energies for heavy projectile-heavy target systems. ☐

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Sinks and sources of plant nutrients

from Peter D. Moore

COMMUNITIES of emergent aquatic plants are known to be among the most productive found in temperate latitudes. Dykyjora (*Photosynthetica* **5,** 329; 1971) examined three such littoral plants, *Phragmites communis, Typha angustifolia* and *Scipus lacustris* and obtained values for their productivity (means for the whole growing season) of up to 25, 39 and 21 g m^{-2} day^{-1} respectively. In part these high values can be explained in terms of the structure and arrangement of leaves; leaf area index is high for all these species and all possess underground rhizomes which act as storage organs and which permit rapid leaf production at the commencement of the growing season. A further factor which contributes to the high productivity of swamp habitats is the normally adequate water supply and the frequently rich provision of nutrients in the water flow.

Tropical swamps also have very high productivity. For example, Thompson (in *The Nile, Biology of an Ancient River* (ed. Rzoska) Dr W. Junk, The Hague; 1976) has estimated that papyrus (*Cyperus papyrus*) swamps in East Africa achieve values of 35 g m^{-2} day^{-1}. As with their temperate counterparts, these plant communities also place high nutrient demands upon their environment and accumulate these nutrients within their living biomass and in the peat which develops from the undecomposed litter.

Gaudet (*Ecology* **58,** 415; 1977) has measured the nutrient uptake capacity of papyrus and has obtained the following values (g m^{-2} yr^{-1}) N, 103; P, 8; K, 130. These figures are similar to published data concerning *Typha latifolia* for P and K, but are twice as high for N. Such very high accumulation rates have subsequently led Gaudet to consider the value of such papyrus communities for the extraction of nutrients and hence the reduction of eutrophication in East African lakes. Nutrient cycles being what they are, however, much of the NPK build up in the biomass is released in litter decomposition. Gaudet has measured the rate of nutrient release, both through decomposition and through the direct leaching of living tissues and has estimated the proportion of the annual nutrient uptake which would enter swamp peat. The figures he obtained are N, 63%; P, 1% and K, 1%. Thus most of the P and K are released back into the environment,

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but much of the nitrogen is taken out of circulation in the peat deposit. As a system for nutrient extraction from eutrophic waters, it might be more efficient to harvest the emergent biomass periodically, but not so frequently that it would result in the undue depletion of rhizome resources. Overcropping is known to weaken some temperate emergent plants; Conway (*J. Ecol.* **30,** 211; 1942) demonstrated such an effect in *Cladium mariscus*, which is reduced in its growth vigour if harvested more frequently than once every four years.

Just as the development of a peatland habitat acts as a sink for the major plant nutrients, so the destruction of a peatland often results in the flushing of such elements back into the environment. Such has been Israel's experience in the Hula Basin to the north of the Lake of Tiberias (Sea of Galilee). Here, extensive swamplands dominated by *Phragmites* have been drained in recent years and the peaty soils have produced rich agricultural land. As in the fens of eastern England, however, there has been a subsidence of the surface, ammounting to about 10 cm yr^{-1}, as a result of the drying and contraction of the peat and its gradual oxidation.

Avnimelech (2nd INTECOL Congress, Jerusalem, 1978) reported a further and more serious problem in the release of large quantities of nitrate from the wasting peat. It is estimated that 20,000 tons of nitrate nitrogen may be released from the area and up to 5,000 tons yr^{-1} have been carried by the Jordan into Lake Tiberias. Such eutrophication, it is feared, could influence the local fishing industry.

Attempts have been made to control floodwater from the Hula Basin, for the greatest movements of nitrate downstream are associated with floodwater. But the most successful measure adopted so far has been the encouragement of denitrification. Alternate wetting and drying of the peat stimulates microbial denitrification and the use of sprinkling has now reduced nitrate leaching into drainage waters by 50%. One of the main crops on the peatland is alfalfa, which is a surprising choice in view of its symbiotic nitrogen fixation. The use of a nitrogen demanding crop species such as rice would seem more appropriate from a nutrient cycling point of view, but is currently precluded by economic considerations. ☐

Faint images revealed

from F. G. Smith

The modern generation of optical astronomers now rely heavily on television and photon-counting techniques, so much so that photography seemed to be rapidly going out of date despite the introduction of sensitive fine grain films such as Kodak IIIa. Competition is, however, a powerful stimulus, and there are now some new photographic techniques giving astronomical photographs which cannot yet be equalled in sensitivity and detail by any new electronic device.

The first step was the hypersensitising of emulsions by soaking in dry nitrogen followed by hydrogen gas. This technique, which gives a large improvement in quantum efficiency, is routinely used in the southern sky survey at the UK Schmidt telescope in Australia. The second step is surprisingly simple, and involves only the method of taking contact copies from the new sensitised plates.

D. F. Malin of the Anglo-Australian Observatory, observed that the photographic grains in faint images are concentrated in the upper layers of the emulsion, while the background fog is distributed throughout the whole depth. He found that contact printing with a diffuse light could be arranged only to reproduce the upper layer, and thereby enhance the signal-to-noise ratio of faint images. He describes the techniques in a note on page 591 of this issue of *Nature* and shows some remarkable results on photographs of the extragalactic nebula Centaurus A and the supernova remnant in Puppis. Faint extensions of both objects are revealed for the first time.

These results are exciting for astronomy, and most gratifying as a further example of a widely useful technical advance stimulated by the needs of pure research.

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More on X-ray bursts

from A. C. Fabian

Spring and autumn are the seasons of the rapid burster. This object produces up to several thousand X-ray bursts per day over intervals of 2-6 weeks in March/April and September/October. Its location near the Galactic Centre means that each burst, of but a few seconds duration, emits in X-rays as much energy as the Sun emits in all wavelengths in one day. Large X-ray detectors on the first High Energy Astronomical Observatory (HEAO-1) were pointed at the rapid burster on 31 March of this year, yielding the light curves shown on page 587 of this week's *Nature*. A collaboration of experimenters from Massachusetts Institute of Technology, University of California at San Diego and Goddard Space Flight Center have found evidence for spectral changes in the decay of rapid bursts.

A spectral softening is observed, but to a less marked extent than appears in other X-ray burst sources, where the burst interval may be many hours. The softening in these other sources may be interpreted as black-body cooling (see for example Swank *et al. Astrophys. J. Lett*, **212,** L73-L76; 1977), leading, with some assumptions as to the luminosities, to a measure of the size of the emitting region. This seems to be relatively constant and is consistent with the surface area of a neutron star (van Paradijs *Nature*, **274,** 650-653; 1978). The region responsible for the optical bursts seen coincident with bursts from MXB 1735-44 (Grindley *et al. Nature*, **274,** 567; 1978) is considerably larger.

There is at yet no detailed theoretical model of the rapid burster, although accretion instabilities in matter flowing onto a neutron star seem a likely candidate for the basis of any such model. The 6 month interval between periods when the burster is observed has also yet to be explained. SAS-3 observations to be published next January (Marshall *et al., Astrophys. J.* in the press) show that a trend in bursting behaviour occurs over the period for which the source is visible. This may imply that the 6 month timescale is intrinsic to the source and not, for example, due to obscuration by a binary companion.

Thermonuclear flashes near the surface of an accreting neutron star have been proposed as the source of the X-ray bursts that are observed to recur on intervals of hours. The flashes are due to a thermal runaway in the helium burning layer of accreted matter. Numerical computations of this process have recently been published by P. Joss (*Astrophys. J.* **225,** L123-L127; 1978) and demonstrate that X-ray bursts can be produced that bear a striking resemblance to the ones observed. The flashes occur at such a depth that heat is transported out fast enough by radiative diffusion that X-ray temperatures are achieved at the photo-

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sphere. Joss' model did not produce flashes if the temperature of the core was too high, although the core was still in thermal equilibrium with the time-averaged heat flow. X-rays are, of course, produced more efficiently by gravitational energy release when the matter is first accreted. The ratio of accretion luminosity to thermonuclear luminosity L_a/L_n must be $\geqslant 30$, for conventional neutron star models, and ~ 300 for Joss' model. $L_a/L_n < 25$ for MXB1759-29 and is $\leqslant 2$ for the rapid burster, if it is assumed that the accretion luminosity emerges as relatively continuous X-rays over the same time interval as that on which bursts are observed. This tends to rule out thermonuclear flashes for the rapid bursts and may prove to be a problem for this model in general. Future work on X-ray bursts will one hopes improve our knowledge of neutron stars and thereby teach us some high energy physics. □

Gene conversion in mitochondria

from D. H. Williamson

THE article on page 577 of this issue by Strausberg and his colleagues from Dallas will rekindle interest in a long-standing problem in the mitochondrial field, namely the mechanism of recombination between mitochondrial DNA molecules.

Mitochondrial genetics is dominated by work on bakers' yeast, and has been ever since the discovery in this organism, some 15 years ago, of the first mitochondrial point mutations conferring resistance to either chloramphenicol or erythromycin. These drugs block protein synthesis on mitochondrial ribosomes, and it happened that these first resistant mutants all mapped close together in the region we now know specifies the 21S mitoribosomal RNA. These mutants were the first tools with which mitochondrial recombination in crosses could be studied, and quite early on, a puzzling phenomenon emerged; in certain crosses, a marked polarity was observed amongst the recombinants, alleles carried by one parent being greatly favoured at the expense of those represented by the other. This polarity was not observed in all crosses, and it was soon realised that any strain could formally be categorised as carrying a hypothetical determinant called omega (ω) in one or other of two configurations, ω^+ and ω^-. Polarity of recombination was observed only in $\omega^+ \times \omega^-$ crosses, and not in either of the other two pairwise combinations. The ω determinant was independent of the mating type of the cell, but an early idea was that its role was a sexual one analogous to Hfr in bacteria and, for a while, the notion of mitochondrial sex was in vogue. However, when mutants in other unlinked genes were discovered, making four-point crosses feasible, it became clear that the analogy with bacterial sex was invalid, and in 1974 the Gif group (Dujon, Slonimski & Weill *Genetics*, **78**, 415; 1974) proposed a comprehensive model to account for all the facts then known. Esentially their model supposes that the mitochondrial DNA molecules in pairs of mating cells undergo repeated rounds of recombination. At the level of the individual molecules, recombination events are considered always to be non-reciprocal. However at most loci, gene conversion can go randomly in either direction, thus generating equal numbers of reciprocal recombinant types in the population as a whole, and obscuring the non-reciprocal nature of the events at the molecular level. At the ω locus, on the other hand, the conversion is seen as always occurring in one direction, effectively favouring alleles of the ω^+ parent, and at the same time unmasking the asymmetric nature of the molecular interactions.

We have since learnt that as far as the events at the ω region are concerned, this model is broadly correct. It has recently been shown that the 21S RNA gene of ω^+ strains carries an insertion of about 1,000 base pairs which is absent from ω^- strains. It is this insertion that seems to be responsible for 'directing' the conversion events in its immediate vicinity. Until now, however, no comparable phenomenon had been discovered in other regions of the genome, and the events at ω were coming to be regarded as something of an odd-ball.

In the light of today's article from the Dallas group, this view becomes a shade less tenable. This group reports on a rather similar state of affairs affecting a recently discovered mitochondrial gene called *var*1. This gene owes its name to the fact that its polypeptide product varies in size in different strains, the extent of this variation amounting to some 4,000 daltons, or around 10% of the polypeptides' molecular weight. The work on *var*1 is of unusual technical interest, since its function is completely unknown, and all the genetic analyses have revolved round the ingenious application of gel electrophoresis to bulk mating populations. With the aid of a variety of genetic and physical mapping procedures, the Dallas group have already precisely located the *var*1 gene on the map, and have shown that the altered sizes of the variant forms of the polypeptide directly reflect changes in the size of the structural gene itself.

The current paper reports on the behaviour of different *var*1 alleles in crosses. It shows, rather clearly, that alterations in the size of the gene (and hence of its polypeptide product) arise as a result of asymmetric gene conversion events involving two small DNA segments (about 36 and 57 base pairs long) which can be independently inserted, by recombination, into alleles which lack them. The very demonstration of these events in a system in which phenotypic expression is restricted to the rate of migration of protein molecules in a gel, is something of a *tour de force*, and fully deserves our admiration. However, the observations have a wider significance in that the phenomena look rather similar to those seen at the ω region. In both cases the gene conversion events occur at high frequencies, and both regions possess insertions presumably responsible for 'directing' the non-reciprocal recombination events at the locus, thereby making them detectable in the overall molecular population.

The main conclusion is clear. Gene conversion is not restricted to the ω locus, and its occurrence at other regions of the genome may have been missed before now simply because of the lack of strategically placed pairs of markers to make it visible. Although no other protein products have been observed to behave like *var*1, there is no lack of evidence for other insertions in the genome (see the Strausberg article for references). Nor is there any lack of models embodying gene conversion to explain some unresolved problems in the transmission genetics of this system. Evidence such as that presented on page 577 begins to invest such models with an air of respectability. □

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The first division

from Clive Lloyd

PROTOPLASTS prepared from tobacco leaves now form the staple experimental diet of investigators studying viral infection, somatic hybridisation, wall synthesis, properties of the plasma membrane—the list goes on—but the intriguing fact goes almost unnoticed that in the process of removing the cell wall, non-dividing leaf cells are stimulated to divide. For instance, remove palisade cells from tobacco

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leaves and few will divide in culture, but remove the wall from such cells with degradative enzymes and about half of the protoplasts go on to divide Nagata & Takebe, *Planta* **92,** 301; 1970), (although it is a common observation that protoplasts must first resynthesise a cell wall before division takes place) It would seem from this that some assembly at the cell periphery is controlling cell division in such a way that its perturbation by enzymes unlocks the potential for growth. This will come as no surprise to animal cell biologists who will be reminded of the ability of some proteases to tweak density-inhibited fibroblasts into another round of division, but compared to animal cells, the role of the cell periphery in controlling cell division has been little studied in plants. A major barrier to studying the plant cell surface with techniques used for animal cells is the cell wall itself; it prevents easy access to the plasma membrane of probes such as proteases, lectins and antibodies, which have helped outline the regulatory role of the cell surface in the division of lymphocytes However, some recent papers hint at potential ways in which the problem can be tackled using protoplasts and a recently adopted wall-inhibitor

Schilde-Rentschler (*Planta* **135,** 177; 1977) reported her work on the role of cell wall formation in the ability of tobacco protoplasts to form callus, finding that the addition of a cellulase preparation to tobacco mesophyll protoplasts inhibits their division. Enzymes used to degrade cell walls are notoriously—and perhaps necessarily—crude preparations. so it is difficult to pin-point cellulose deposition as a prerequisite for division when so many other sites are probably affected simultaneously. However, by adding the disaccharide, cellobiose (believed here to be inhibiting cellulase activity), to protoplasts cultured in the presence of the enzyme mixture. Schilde-Rentschler was able to restore to protoplasts the capacity to divide, thereby claiming a direct involvement for cellulose formation in allowing division to take place. It is unclear from this whether cellulose biosynthesis triggers cell division or whether cellulosic wall formation simply provides the mechanical framework which allows the division of protoplasts stimulated to do so during the earlier event of wall removal. A series of papers from Meyer *et al.* favours the latter possibility, suggesting that wall formation, although required for organised cytoplasmic division, may be unnecessary in the events which lead to nuclear division.

To get protoplasts to divide at all in culture was, originally, far from straightforward and most workers use variations of media found successful for tobacco leaf protoplasts Unlike such media which osmotically cushion the fragile protoplasts with sugars, Meyer & Abel (*Planta* **123,** 33; 1975) used instead, salts for maintaining osmotic strength. In this alternative system, tobacco protoplasts regenerate only a pseudo-wall which differs from wall regenerated in sugar medium by being discontinuous (patches of wall-less membrane exist which permit cell fusion in the presence of polyethyleneglycol), non-rigid (does not resist plasmolysis) and lacking in some typical wall enzymes However, the regeneration of such a pseudo-wall still permits a form of cell division to continue for 2-3 rounds Cytoplasmic division can, therefore, take place in the absence of a rigid cell wall, but related papers (*Planta* **125,** 1; 1975; *Planta* **142,** 11; 1978) showed that the quality of those divisions is abnormal. In more normal circumstances, cells would divide by coalescence at the equator, of vesicles containing materials which go to form the separating wall; but with reduced competence to form wall in the salts medium, the empty vesicles fuse and perforate the daughter cells in a manner likened to the budding of yeasts.

Now, in their most recent paper, Herth and Meyer (*Planta* **142,** 253; 1978) go one step further and prevent even pseudo-wall formation by using a reversible inhibitor of cellulose formation 2,6-dichlorobenzonitrile. In this case, no cytoplasmic division is observed, but nuclear division continues In fact, Meyer and Herth report that within 6 days of culture, as many as 90% of the protoplasts have performed at least one mitosis, forming, two-, four-, and multi-nucleate cells The fact that nuclear division occurs in the presence of this inhibitor not only confirms that nuclear and cytoplasmic division are seperable, but that the sequence of events leading to nuclear division continues to operate in the absence of extensive deposition of cellulose The mitotic activity of wall-less protoplasts has been reported previously by several authors, but the potential interest of the present system is that the wall-less state can be maintained by the presence of inhibitor, thereby allowing any possible role of the plasma membrane in regulating mitosis to be examined. Further quantitative data on the contribution that nuclear division, as opposed to cell fusion, makes to the polynuclear state, would be welcomed, as would a greater understanding of how the inhibitor works. Whether or not these particular experiments stand the test of time, their general motive seems right: that we should seek novel approaches to investigate the role of the cell surface in regulating plant cell growth ☐

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Can magnetic order and superconductivity coexist?

from B. R. Coles

IT has long been recognised that in rough approximation magnetism and superconductivity are sworn enemies This is shown not only in the ability of an applied magnetic field to destroy the superconductive state but also in the strong suppression of T_c, the transition temperature, by additions of magnetic-moment bearing atoms, where simple solutes have little effect When enough magnetic atoms have been added to destroy superconductivity it is often succeeded at low temperatures by a state in which the solute moments couple by way of the (now normal) conduction electrons In random solid solutions the resultant state cannot easily be one of long-range magnetic order—it is now generally called a spin glass but there have recently been discovered some atomically well-ordered intermetallic compounds containing a fraction of moment-bearing atoms (rare earths with partly filled 4f electron shells) where tendencies towards magnetic order and towards superconductivity are both present. These are compounds of the form RE Mo_6S_8 (Fischer *et al Solid State Commun* **17,** 721; 1975)—the Chevrel phases—and of the form RE Rh_4B_4 (Matthias *et al. Proc natn Acad. Sci U S A.* **74,** 1334, 1336; 1977) where RE is a rare-earth metal In the latter group of compounds superconducting transitions were found for RE given by Y, Nd, Sm, Er, Tm or Lu, but it was later found that in the Er compound (Fertig *et al Phys Rev Lett.* **38,** 987; 1977) the superconductivity setting in at 8 7 K disappears with the formation of a state of magnetic long-range order below 0 9 K. The presence of magnetic order was inferred from a pronounced λ type specific heat anomaly and an accompanying susceptibility peak, neutron diffraction experiments have

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since demonstrated (Monckton *et al* *Phys. Rev. Lett.* **39**, 1164; 1977) that the order is ferromagnetic.

It is impressive (and to some people surprising) that these properties of Er Rh_4B_4 can be given a convincing discussion (Jarlborg *et al. Phys Rev. Lett* **39**, 1032; 1977) in terms of a calculated electronic structure. That any reasonable form of band structure calculation for a compound containing three types of atom and eighteen atoms per crystal unit cell can now be carried out is a testimony to the enormous progress made in such calculations by the combination of intelligent choices of calculational schemes and large amounts of computing capacity The superconductivity seems to be associated with a high density of 4d-electron states (per unit energy) at the Rh atom sites, just as in the superconductivity of pure V and Nb (although for other reasons not in pure Rh), while the rather small density of states for 5d electron states on the Er sites, provides a magnetic coupling like that in pure Er between the local 4f moments on these sites This, while not as strong as that between the larger 4f moments which completely suppresses superconductivity in favour of magnetic ordering in Gd Rh_4B_4, does finally, below 0.9 K, enable magnetic order to appear.

It seems not unreasonable that a long range magnetic order of ferromagnetic character should be incompatible with superconductivity since there is no sharp separation between two types of conduction band electrons (4d-like on Rh and 5d-like on Er), and the long-wave-length (zero wave-vector) spontaneous parallel spin pairing which is the essence of ferromagnetism cannot coexist with the Fermi surface antiparallel pairing which is the essence of normal superconductivity. The other forms of pairing which parallel the superfluidity of liquid ^3He are, as yet, only gleams in the eyes of theoreticians

In other groups of compounds, the Chevrel phases RE Mo_6S_8 and RE Mo_6Se_8, there has, however, been some evidence for the coexistence of superconductivity and long range magnetic order Thus, the La Jolla group (see the review by Maple in *Transition Metals*, 655; 1977 (Inst Physics Conf Series No 39, 1977)) have found specific heat anomalies below T_c in RE Mo_6Se_8 for Sm, Gd, Tb, Dy and Er, which they ascribe to the formation of a state of magnetic long-range order, probably antiferromagnetic, within the superconducting phase field In view of the important theoretical implications of such a coexistence (in formal terms a divergence in the wave-vector dependent susceptibility χ (q) at some antiferromagnetic wave vector q_0 while χ (0) goes to zero) it is of great interest that it has proved possible very recently to show the antiferromagnetism of the Dy compound unambiguously by neutron diffraction techniques.

In a recent paper (Monckton *et al. Phys Rev. Lett.* **41**, 1133, 1978), research workers from the pioneer Geneva group, from Bell Labs and from Brookhaven have used the Brookhaven neutron scattering facilities to examine a specimen of Dy Mo_6S_8 down to a temperature of 50 mK, and have shown conclusively that this material (which becomes superconducting at 2.05 K) develops long-range antiferromagnetic order at 0 4 K. Conventional powder diffraction patterns taken above and below this temperature, together with high resolution scans of certain peaks, have permitted the identification of the detailed magnetic structure and yielded estimates of the ordered moments, which show some suppression by crystal field effects from the free Dy^{3+} ion values

The coexistence of superconductivity and antiferromagnetic long-range order is now proven; a theoretically satisfactory account of such coexistence has yet to be produced ☐

A star called M

from S. Jocelyn Bell Burnell

IN THE good old days (that is more than 10 years ago) people believed that neutron stars would only be detectable (if at all) through their steady, thermal optical emission Calculations of the expected brightness are difficult to perform realistically; heat is generated in bizarre circumstances by friction between charged particles and a neutron superfluid in the interior of the star and there is heat loss by radiation from the surface, but the character of this radiation is drastically modified by the presence of a magnetic field of about 10^{12} gauss Indications are that the surface temperature should be about 10^6 K and that the most readily detectable neutron stars would, optimistically, be within the grasp of the world's largest telescopes or, more realistically, might be just beyond them

In the event, however, neutron stars were detected much more readily by radio astronomers (as pulsars) and soon afterwards by X-ray astronomers who concluded that many of the strong X-ray sources in our Galaxy were powered by a neutron star's gravity Radio astronomers now know of some 300 pulsars. All pulse regularly, and their periods range from 33 ms to about 4 s. But only two of these three hundred have been detected at optical wavelengths. They are two of the most rapid pulsators—those in the Crab and Vela nebulas—and it was through their pulsed optical emission that the star responsible was ultimately identified in each case

The Vela pulsar proved difficult to detect at optical wavelengths At one stage astronomers despaired of detecting pulses and looked for a star whose emission might be accounted for as steady thermal radiation of a hot neutron star On these grounds Lasker suggested as candidate a star he called M That star called M turned out to be the pulsar

Now events have taken another interesting turn bringing us back, almost, to where we started The group that discovered the optical pulsations from Vela report (Peterson *et al Nature* **276**, 475, 1978) that some 50% of the optical emission from this star is unpulsed. A series of frames taken during the pulsation cycle of the star shows quite clearly the double-pulsed profile which is its signature, and some residual light during the 'off' part of the pulsar's duty cycle. However, there is too much steady emission for it to be thermal emission from the surface of the star and they are forced to consider other possibilities Perhaps it is the pulsar emission reflected off a cloud of gas or off the surface of the star; perhaps there are several sources of emission around the star which blur together; perhaps there are very broad pulses which fill the whole cycle

They find a similar but perhaps less dramatic effect for the Crab pulsar. Here again there were technical difficulties, but this time it was the detection of the weak, steady emission in the face of the short but bright flash from the pulsar Whereas the Vela pulsar needed 8 hours' observation with the 3.9 m Anglo-Australian Telescope, the Crab pulsar needed only 10 minutes' observation with the telescope stopped down to approximately half aperture! The conclusion is that the Crab too has steady optical emission, and although it's a smaller proportion of the total emission once again it is too copious to be accounted for by a thermal mechanism

It will be interesting to see whether the latest generation of EUV and soft X-ray astronomy satellites can observe a similar effect Meanwhile these incredible stars continue to puzzle, and occasionally to surprise Less surprising will be the inevitable spate of papers which endeavour to explain this latest result ☐

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review article

A gene complex controlling segmentation in *Drosophila*

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The bithorax gene complex in Drosophila *contains a minimum of eight genes that seem to code for substances controlling levels of thoracic and abdominal development. The state of repression of at least four of these genes is controlled by* cis-*regulatory elements and a separate locus (Polycomb) seems to code for a repressor of the complex. The wild-type and mutant segmentation patterns are consistent with an antero-posterior gradient in repressor concentration along the embryo and a proximo-distal gradient along the chromosome in the affinities for repressor of each gene's* cis-*regulatory element.*

THE segmentation pattern of the fly provides a model system for studying how genes control development. The phylogeny, although not the ontogeny, of that pattern is reasonably well understood. Flies almost certainly evolved from insects with four wings instead of two and insects are believed to have come from arthropod forms with many legs instead of six. During the evolution of the fly, two major groups of genes must have evolved: 'leg-suppressing' genes which removed legs from abdominal segments of millipede-like ancestors followed by 'haltere-promoting' genes which suppressed the second pair of wings of four-winged ancestors. If evolution indeed proceeded in this way, then mutations in the latter group of genes should produce four-winged flies and mutations in the former group, flies with extra legs. In *Drosophila*, not only have both types of mutation been observed, they have been shown to involve a single cluster of pseudoallelic genes known as the bithorax complex (BX-C)[1-7]. During evolution a tandem array of redundant genes presumably diversified by mutation to produce this complex[1,4]. During development the BX-C genes seem, on the basis of evidence to be summarised in this article, to control much of the diversification of the organism's thoracic and abdominal segments. It is as if during ontogeny the BX-C genes recapitulate their own phylogeny.

Each of the wild-type thoracic and abdominal segments has a unique pattern of differentiated structures (Fig. 1*a*) which constitutes a morphologically defined state or 'level of development' (L). Each BX-C mutant phenotype can be described in terms of the degree to which one or more segments, or portions thereof, are transformed from one such level to another. As shown in Fig. 1*b* a striking feature is that the metathoracic (MT) and first three abdominal segments (AB1, AB2 and AB3) can approach or achieve almost all segmental levels of development from a mesothoracic level (LMS), or 'primitive' level[1], to a third abdominal level (LAB3), depending upon the genotype. The wild-type allele of each BX-C gene (Fig. 2) will be assumed to code for a BX-C substance (S) which controls one or more components of an intersegmental transformation (Table 1). The various BX-C substances are presumed to act indirectly by repressing or activating other sets of genes which then directly determine the specific structures and functions that characterise a given segment. On such a model the level of development which a segment achieves depends upon the particular array of BX-C substances elaborated in that segment. When the wild-type and mutant phenotypes are interpreted in accordance with this model, it can be seen from Fig. 1*b* that the attainment of any level more

Table 1 Summary of the roles of BX-C substances (S) in controlling specific types of body segment transformations and specific structures in one or more segments of the larva

Gene	Substance	Segmental transformation	Larval structures affected	Genotypic comparisons (from Fig. 6)
bx$^+$	S_1*	LAMS → LAMT		
pbx$^+$	S_2	LPMS → LPMT		
Ubx$^+$	S_0*	LMS → LMT	DLT	*a* vs *b*
				f vs *h*
bxd$^+$	S_3	LMS → LAB1	DLT†, VP, KO, VSB	*g* vs *h*
lab-2$^+$	S_4	LMS → LAB2		*b* vs *c*
			DLT, KO, VSB	*c* vs *h*
lab-3$^+$	S_5	LAB2 or LMS → LAB3		*e* vs *f*
lab-5$^+$	S_7	LAB4 or LMS → LAB5		
lab-8$^+$	S_x	LAB7 or LMS → LAB8	DLT, PSP, CP, VP‡, KO‡	*a* vs *d*

* Substance, S_1, was originally postulated to be coded for by either *bx*$^+$ or *Ubx*$^+$ (ref. 4); in this article S_1 is assigned to *bx*$^+$ and S_0 to *Ubx*$^+$.
† Comparison of genotype *e* and *f* indicates that *bxd*$^+$ and/or *lab-2*$^+$ may be responsible for the continuity of dorsal tracheal trunk in AB1.
‡ Suppression of VP and KO may result from the presence of *lab-8*$^+$ and/or one or more *lab* genes located between *lab-3* and *lab-8*.

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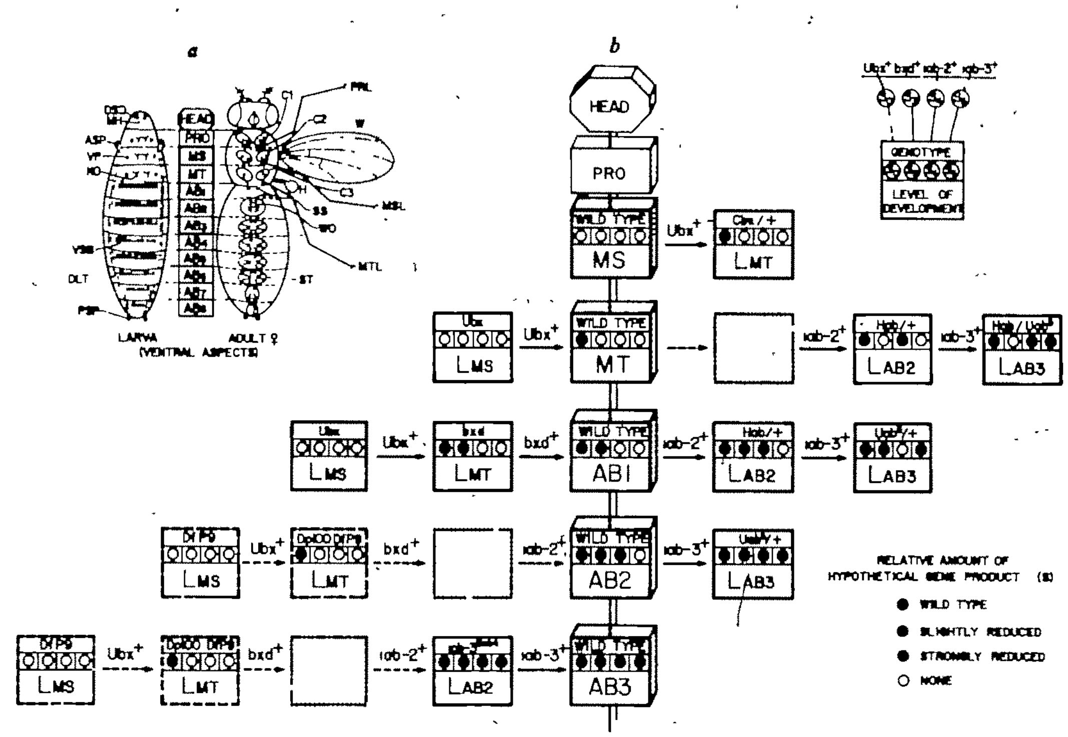

Fig. 1 Genetic control of segmental levels of development in thoracic and abdominal segments. *a*, Anatomy of third instar larva. Head: dorsal sense organs (DSO) and protruding portion of mandibular hooks (MH). Thoracic segments: each bears a pair of Keilin's organs[13] (KO), three sensory hairs in a depression; ventral pits (VP) and ventral setal bands (VSB) with fine tooth-like setae. Abdominal segments: VSB with coarse tooth-like setae; KO and VP lacking. Each dorsal longitudinal trunk (DLT) terminates in an anterior (ASP) and posterior (PSP) spiracle. Anatomy of adult female. Thoracic segments: coxae (C1, C2 and C3) of prothoracic (PRL), mesothoracic (MSL) and metathoracic (MTL) legs; wing (W) and haltere (H), being dorsal, are shown in dotted outline. Abdominal segments: AB1 to AB7 bear sternital sensillae (SS); AB2 to AB7 bear bristled sternites (ST); AB2 has a raised lump, or Wheeler's organ[10] (WO) *b*. Levels of development (L), achieved in wild-type segments (vertical array of connected boxes) and approached in mutant segments, are depicted in panels containing genotype, phenotype and status with respect to relative amounts of hypothetical BX-C substances, S_0, S_3, S_4 and S_5, products of the wild-type alleles of Ultrabithorax (*Ubx*), bithoraxoid (*bxd*), infra-abdominal-2 (*iab-2*) and infra-abdominal-3 (*iab-3*). Panels to the left of wild type depict hemizygous genotypes for hypomorphic or amorphic mutants, the designated chromosome being opposite *Df-P9* (see Fig. 2) in all cases. For simplicity, a slight transformation of the posterior region of AB1 towards LMS in the *bxd* hemizygote is ignored. Panels to the right of wild type involve heterozygotes for hypermorphic (dominant-constitutive) mutants, Contrabithorax (*Cbx*), Hyper-abdominal (*Hab*) and Ultra-abdominal-5 (*Uab*^{5}). Panels in dotted outline represent theoretically possible LAB1 transformations for which definitive mutant genotypes are not available. Panels in dashed outline are genotypes dying in the first instar. All other genotypes shown survive to the adult stage except *Ubx* hemizygotes which die in either third instar or early pupal stages.

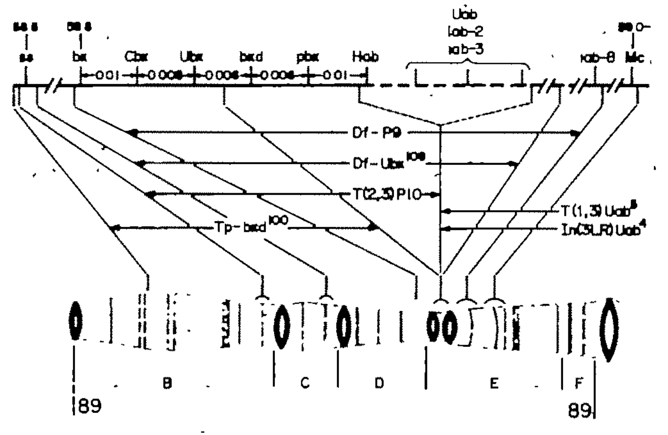

Fig. 2 Correlation of the linkage and salivary gland chromosome maps of BX-C and immediately surrounding regions in the right arm of the third chromosome. The solid line portion of the linkage map is based on half-tetrad[6], as well as on conventional, linkage analysis. The dashed line portion is based on unpublished cytogenetic studies of the designated rearrangements, the relative order of *Hab*, *Uab*, *iab-2* and *iab-3* with respect to each other being uncertain. The complex spans approximately 0.05 to 0.1 centimorgan and lies within the region of two heavy doublet structures and possibly an adjoining faint band of section 89E. Not shown is a spontaneous dominant mutant, Miscadestral pigmentation (*Mcp*), recently found by M. Crosby, which maps between *Hab* and Microcephalus (*Mc*)[15]. *DF-P9* in its interactions with BX-C mutants and rearrangements acts as if it is deleted for all of the BX-C genes. *Df-P9*/+ has reduced male pigmentation in AB5 and AB6, is sterile, and has deformed genitalia in both sexes, which on the basis of other interactions and of larval studies (Fig. 6*d*) behaves as a deficiency extending from *bx* to *iab-3*, associated with a weakened dominance of genes to the right of *iab-3*. *Mc* is apparently a very small tandem duplication which partially restores fertility when *trans* to *Df-P9* but does not otherwise modify the *Df-P9*/+ phenotype. *T(2; 3)P10* is an insertional translocation of the designated region of 89 to section 29C of chromosome 2; the breakage point in 89E is likely on genetic grounds to lie between *pbx* and *iab-2* and to be accompanied by polar position effects that weaken the dominance of *iab-2*$^+$ and *iab-3*$^+$. *Tp(3)bxd*^{100} is a transposition of the designated 89 region to 66C of chromosome 3L; the breakage point lies between *Ubx* and *bxd* and is accompanied by a weakened dominance of *bdx*$^+$ and *pbx*$^+$ (ref. 1) but not, as far as can be detected, of genes to the right of *pbx*. *T(1; 3)Uab*^{5} is a reciprocal translocation with a breakage point in section 1E of the X chromosome; a synthetic deletion analysis indicates that the *Uab*^{5} phenotype is associated with the distal portion of the translocation; that is, the breakage point in 89E lies to the left of *Uab*^{5} and falls either to the left or right of *lab-2*$^+$. *In(3LR)Uab*^{4} is a complex inversion with additional breaks in 80C and in or near 85A or 3R. Analysis of X-ray induced revertants ot *Uab*^{4} indicates that the breakage point in 89E probably lies just to the left or right of *iab-2* and is associated with a weakened dominance of *iab-3*$^+$ (designated in Fig. 1 *iab-3*^{Uab4}) and possibly of *iab-2*$^+$, as well. *Hab* was originally designated as the F locus of the complex[7]; and has also appeared in the literature as Contrabithoraxoid (*Cbxd*). The first two *Uab* mutants found, *Uab* and *Uab*^{2}, although cytologically apparently normal, are associated with a reduction in crossing over within the complex and are accompanied by position effects or point mutations at other loci within BX-C. Their dominant phenotypes resemble that of *Uab*^{4} much more closely than that of *Uab*^{5}. Cellular determination of *Uab* has been extensively studied by Kiger and Davis[20,21].

advanced than LMS is a stepwise process in which each step requires the presence of a specific BX-C substance. In keeping with the model, all of the genotypes involving recessive loss of function lie to the left or LMS side of the wild-type segments while those involving dominant gain of function lie to the right or LAB3 side of those segments.

Segment MT transforms towards LMS even in larval stages of homozygotes[1] and hemizygotes for Ultrabithorax (*Ubx*). The most prominent feature is the occurrence of an extra set of spiracles on MT (and also AB1) (Fig. 3*a*) compared to the usual single set on MS. Although such animals die in late larval or early pupal stages, adult *Ubx*/*Ubx* cuticular tissue in MT has been shown by somatic mosaic analysis to transform autonomously towards LMS[4,8]. A substance, S_0, effecting LMS → LMT, will be assumed to be the *Ubx*$^+$ product. The inability of MT to achieve LMT in *Ubx* hemizygotes or homozygotes is then consistent with the expected reduction in amount of S_0 in that segment. The adult MT cuticle, but not the larval tracheal system, strongly transforms toward LMS in a double mutant combination involving bithorax-3 (*bx*^{3}) and postbithorax (*pbx*), notably producing a four-winged fly[5,8]. Substances S_1 and S_2 are assumed to be the products of *bx*$^+$ and *pbx*$^+$, respectively (Table 1), and to be involved in effecting LMS → LMT; however, for the sake of simplicity, they are omitted from Fig. 1*b*. The wild-type phenotype, on these assumptions, corresponds to that expected if *bx*$^+$, *Ubx*$^+$ and *pbx*$^+$ are repressed in MS and derepressed in MT.

Segment MS takes on MT characteristics in flies heterozygous or homozygous for Contrabithorax (*Cbx*), the most conspicuous effect being a partial conversion of wings into halteres[5,9]. *Cbx* is known to regulate *Ubx*$^+$ in a *cis*-dominant fashion; that is, MS transforms towards LMT in *Cbx* +/+ *Ubx* but is virtually wild type in *Cbx Ubx*/+ +[2,5]. *Cbx* presumably damages a regulatory element adjacent to *Ubx*$^+$ causing S_0 to be made constitutively in MS but in a relatively reduced amount compared with that made in MT when *Ubx*$^+$ is in the wild-type configuration.

Segment AB1 transforms primarily towards LMT in hemizygotes for bithoraxoid (*bxd*), producing flies with one or two MT-like legs on AB1, and on rare occasions a tiny haltere-wing as well[5]. This phenotype has been accounted for by postulating that *bxd*$^+$ codes for a substance, S_3, effecting LMS → LAB1[4]. Evidence to be represented later suggests that *Ubx*$^+$ is derepressed not only in MT but also in AB1 and segments beyond. Hence, in the *bxd* hemizogote, there is presumably enough S_0 in AB1 to transform that segment towards LMT. That AB1 remains at LMS in the *Ubx* hemizygote (Fig. 3*a*) is expected since *Ubx* is known to exert a strong polar effect on *bxd*$^+$ (ref. 1); that is, neither S_0 nor S_3 is expected to be made in sufficient amounts to transform AB1 appreciably towards LMT or LAB1. In wild type, S_0 as well as S_3 is presumably being produced in AB1; although S_3 function may override that of S_0, a more attractive possibility is that LAB1 actually corresponds to a mosaic of structures and functions controlled jointly by S_0 and S_3.

Segments MT and AB1 approach LAB2 in heterozygotes for a second type of dominant regulatory mutant, Hyperabdominal (*Hab*), producing flies with missing halteres and/or MT legs and with an AB2-type of sternite pattern[10,11] on MT and AB1 (Fig. 4*b*). These effects are variably expressed within a fly even though the penetrance of *Hab*/+ exceeds 99% in certain backgrounds.

Cis-trans studies involving all possible double mutant combinations between *Hab* and *bx*^{3}, *Ubx*, *bxd* and *pbx* indicate that *Hab* does not derepress the wild-type alleles of any of these genes. *Hab* will be assumed to cause partial derepression of a wild-type infra-abdominal-2 (*iab-2*$^+$) gene, whose coding product, S_4, effects LMS → LAB2. On this assumption, the wild-type phenotype is that expected if *iab-2*$^+$ is repressed in MT and AB1 and derepressed in AB2. Similarly, the *Hab*/+ phenotype is that expected if *iab-2*$^+$ is partially derepressed in MT and AB1 and fully derepressed in AB2.

The failure of MS to be modified in *Hab*/+ requires explanation. The *Hab* mutation presumably damages a regulatory element adjacent to *iab-2*$^+$ in such a way as to reduce its affinity for a repressor. If there is an entero-posterior concentration gradient in that repressor, then conceivably the threshold concentration for keeping *iab-2*$^+$ repressed in the *Hab* chromosome is exceeded in MS but not in MT and segments beyond MT.

Segments AB1 and AB2 partially transform toward LAB3 in adults heterozygous for Ultra-abdominal-5, *Uab*^{5}, a third type of dominant constitutive mutant, inseparable from an X-3 translocation (Fig. 2). The transformation is most easily seen in the ventral regions of AB1 and AB2 which develop sternite patterns approaching those found in AB3 (Fig. 4*c*). The effect on AB2 can be accounted for if *Uab*^{5} derepresses a gene, *iab-3*$^+$, which codes for a substance, S_5, effecting LAB2 → LAB3. The wild-type pattern then corresponds to that expected if *iab-3*$^+$ is repressed in AB1 and AB2, but derepressed in AB3. The penetrance of *Uab*^{5}/+ is complete but the transformation of AB1 towards LAB3 is variable with that segment often progressing only towards LAB2. If, as certain larval findings discussed below suggest, *iab-2*$^+$ is normally weakly derepressed in AB1, then the presence of S_4 in AB1 even in subthreshold amounts may facilitate the transformation to LAB3 in *Uab*^{5}/+. Another possibility is that the *Uab*^{5} rearrangement in some way partially derepresses *iab-2*$^+$ as well as *iab-3*$^+$; however, although MT transforms to LAB2 in *Hab*/+, MT is not modified in *Uab*^{5}/+.

Segment MT is able to approach LAB3, along with AB1 and AB2, in *Hab*/*Uab*^{5} animals (Fig. 4*d*). Transformation to LAB3 in MT thus seems to require two sequential steps: LMS → LAB2 → LAB3, the former controlled by S4 and the latter by S_5. The observed transformation of all three segments towards LAB3 is readily accounted for, since both S_4 and S_5 are expected to be produced in MT, AB1 and AB2 of *Hab*/*Uab*^{5}.

Additional evidence for an *iab-3*$^+$ gene comes from an analysis of another rearrangement-associated *Uab* mutant, *Uab*^{4}. When heterozygous, *Uab*^{4} shows a dominant gain of function in which AB1 partially transforms towards LAB2. When hemizygous, or opposite *T(2; 3)P10* (Fig. 2), *Uab*^{4} exhibits a new type of recessive loss of function; namely, the sternites of not only AB1 but AB3 to AB6, inclusive, transform towards LAB2 (Fig. 3*c*). These latter effects are attributed to a reduction in the amount of S_5 which would be expected if the *Uab*^{4} and *T(2; 3)P10* rearrangements are accompanied by position effects on *iab-3*$^+$.

Gonads, normally located in AB5, are absent in *Uab*^{4} hemizygotes, suggesting that BX-C genes control mesodermal as well as ectodermal tissues. Gonadal examination of *Uab*^{4} genotypes was prompted by the report of gonadal defects in homozygotes for certain *Uab*-like mutants of the homologous 'E-locus' complex of Bombyx (see review by Tazima[12]).

Segment AB4 transforms strongly towards LAB5, both in pigmentation and bristle patterns in heterozygotes and homozygotes for Miscadestral pigmentation (*Mcp*), a dominant mutant recently discovered by M. Crosby (unpublished). Analysis of X-ray induced revertants indicates that *Mcp* probably derepresses a gene, *iab-5*$^-$, which codes for a substance, S_7, effecting either LAB4 → LAB5 or LMS → LAB5.

A new dimension has been added to the analysis of BX-C with the discovery that a homozygous deficiency for the entire complex, *Df-P9* (Fig. 2) survives to the late embryonic or early first instar stage and exhibits a transformation of MT and all eight AB segments towards LMS. Pairs of Keilin's organs[13] (KO), ventral pits (VP) and thoracic-type ventral setal bands (VSB) appear on those segments (Fig. 5*a* and 5*b*) and in place of a continuous dorsal longitudinal tracheal trunk (DLT) each segment bears a section of DLT terminating presumably in an incipient anterior spiracle (ASP)[14] (Fig. 5*c*, vs. *d*); finally, the ventral nerve cord tends to retain its primitive embryonic pattern in that it extends to segment AB6 or AB7 instead of foreshortening to segment AB3 or AB4 as in wild type. Tiny

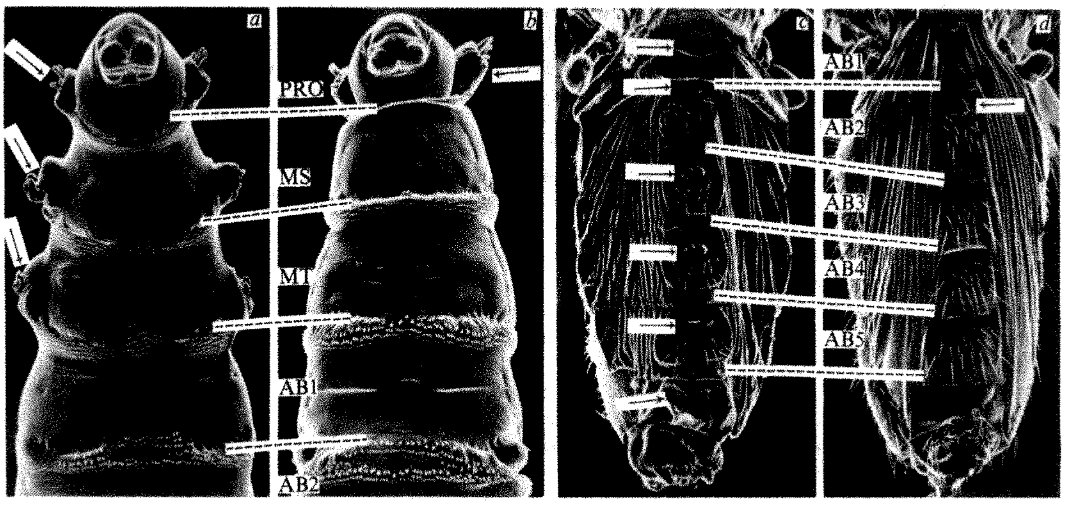

Fig. 3 Mutant phenotypes resulting from recessive loss of function compared with wild type. See Fig. 1(*a*) for legend. *a*, Late third instar larva hemizygous for *Ubx* (*Ubx/Df-P9*). Note extra pairs of anterior spiracles (ASP) (arrows) on MT and AB1, protruding into MS and MT, and thoracic type of ventral setal bands (VSB) on AB1 compared with abdominal type on AB2. *b*, Corresponding stage of homozygous wild-type (Canton-S) larva. Note single pair of ASP (arrow) which arises in MS but protrudes into the region of the prothorax (PRO). Note setae of VSB are coarse in AB1 and AB2, fine in MS, and intermediate in MT. (Specimens *a* and *b* were fixed in hot water and photographed with SEM, X40. *c*, Ventral aspect of male abdomen of genotype *In(3LR)Uab*^{4}*/T(2; 3)P10* (see Fig. 2). Note presence of WO (arrows) and sternital bristle pattern of AB2 type on segments AB1 through AB5. Gonads are rudimentary in specimens of this genotype or lacking entirely in *Uab*^{4}*/Df-P9*. *d*, Corresponding region of adult wild-type (Canton-S) male. Note that AB1 lacks a sternite and that only AB2 (arrow) has WO. (Specimens *c* and *d* were unfixed and photographed with SEM, ×50.)

chitinised plates (CP), resembling rudiments of mandibular hooks (MH), occur in AB8 and imply a head-like rather than LMS transformation of one or more of the embryonic segments that normally fuse to form AB8.

In order to delineate regional differences in morphological functions within BX-C, hemizygotes for deficiencies of portions of BX-C have been constructed. Figure 6 displays stylised phenotypes associated with genotypes hemizygous for five such deficiencies (*b–f*), for *Df-P9* (*a*), for *bxd* (*g*) and for wild type (*h*).

A direct demonstration that *Ubx*$^+$ effects LMS → LMT can be seen by comparing genotypes *a* and *b*, which lack all BX-C genes except that *b* retains one dose of *Ubx*$^+$ (and *bx*$^+$). All nine segments from MT to AB8 remain at LMS in *a* and attain LMT in *b*; that is, in *b* they acquire a pattern of attributes unique to the larval MT (namely, a continuous DLT, VP, KO and thoracic-type VSB). Furthermore *Ubx*$^+$ behaves as if derepressed not only in MT and AB1, but in the remaining abdominal segments as well. That many other BX-C genes share with *Ubx*$^+$ the ability to establish continuity of DLT is evidenced by the presence over several segments of continuous DLT in larval genotypes, *d*, *e* and *f*, which lack *Ubx*$^+$ but have one or more other BX-C genes still present.

All abdominal segments approach, but do not quite achieve, LAB1 in animals of genotype *c*, which is a hemizygote for *bxd*$^+$ (and *pbx*$^+$) as well as *Ubx*$^+$. Such animals are seen to have most of the characteristics of wild-type AB1 (absence of ASP and VP and presence of DLT and VSB, but incomplete suppression of KO). Failure of *bxd*$^+$ to effect a perfect transformation to LAB1 is not understood. One possibility is that *iab-2*$^+$ and *bxd*$^+$ are

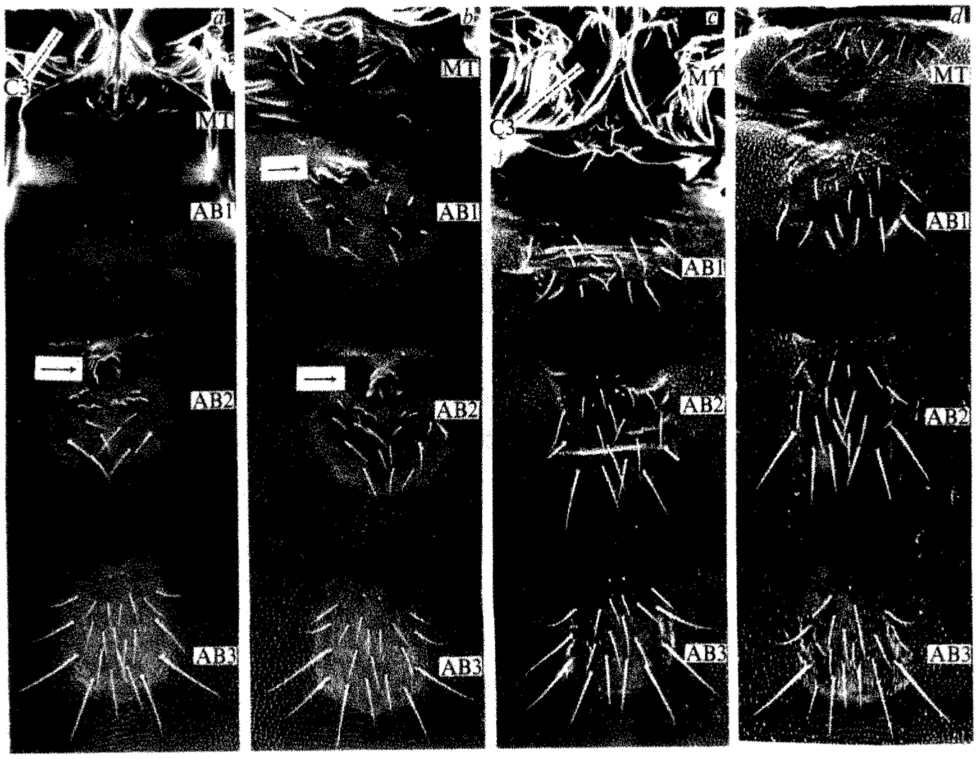

Fig. 4 Mutant phenotypes resulting from dominant gain of function compared with wild type. Mid-ventral cuticular regions of adults female with MT, AB1, AB2 and AB3 approximately parallel to one another. (All specimens unfixed and photographed with SEM.) *a*, Wild type. A portion of coxa-3 (C3) is visible on MT; AB1 lacks a sternite; AB2 has WO (arrow) and a reduced (compared to AB3) sternital bristle pattern, ×80. *b*, *Hab/+*. The specimen had four legs and no halteres. WO (arrows) and bristle pattern on MT and AB1 approach those of Ab2, ×80. *c*, *Uab*^{5}*/+*. C3 resembles that of wild type. AB1 and AB2 sternites lack WO and approach in bristle pattern that of AB3, ×90. *d*, *Hab/Uab*^{5}. The specimen had four legs and no halteres. Sternites on MT, AB1 and AB2 have little or no trace of WO and approach in their bristle patterns that of AB3, ×80.

both involved in the suppression of KO and that *iab-2*$^+$ is weakly derepressed in AB1 (depicted in Fig. 6*h* by indicating a strongly reduced amount of S_4 in that segment of wild type). Complete suppression of KO can occur in genotypes which lack full *bxd*$^+$ function; namely, *f* and *g*. In such cases, it seems likely that *iab-2*$^+$ and possibly *iab-3*$^+$ are able to bring about suppression of KO when fully derepressed.

Suppression of VP seems to be exclusively controlled by *bxd*$^+$, these organs being absent from abdominal segments only when *bxd*$^+$ is present, as in genotypes *c* and *h*. It is therefore directly evident from *h* that *bxd*$^+$ is normally derepressed not only in AB1, but in all segments posterior to AB1.

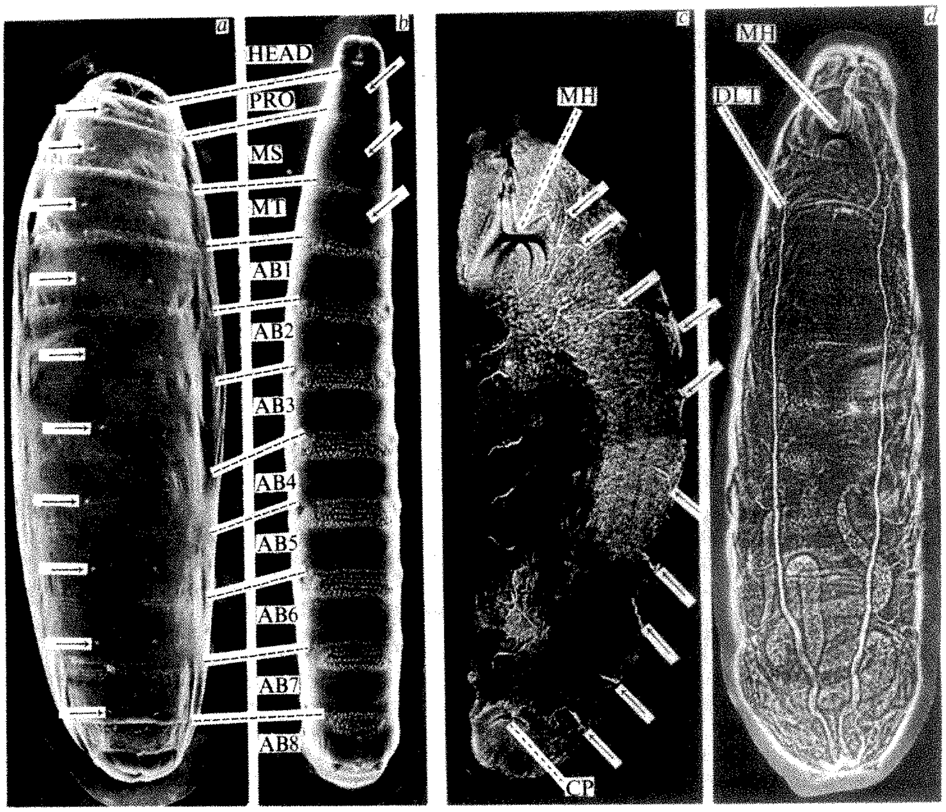

Fig. 5 Comparison of cuticular and tracheal patterns in homozygotes for a deletion of BX-C (*a* and *c*) compared with those of wild type (*b* and *d*). See Fig. 1 for legend. *a*, *Df-P9* homozygote. Animals die within the egg or after partly emerging from egg membranes. Note thoracic-like VSB, KO (arrows) on segments AB1 through AB7, as well as on thoracic segments; AB8 has a reduced VSB. KO not visible in AB8 of specimen shown but are visible in whole mounts examined under phase contrast, ×160. *b*, Wild-type first instar larva. Note more prominent VSB on abdominal vs. thoracic segments, with VSB on MT somewhat intermediate between those on MS and AB1. KO (arrows) are restricted to thoracic segments as are also VPs, which are not resolved at the magnification shown, ×60. (Specimens *a* and *b* were fixed in hot water and photographed with SEM.) *c*, Whole mount of mature embryo of *Df-P9* homozygote. Note separate tracheal sections (arrows) of DLT occur in each segment from MS through AB8, and tiny chitinized plates (CP) in AB8. *d*, Whole mount of wild-type first instar larva. Note continuous DLT. (Specimens *c* and *d* mounted in Zeiss W15 medium and photographed under phase contrast, ×120.)

The larval effects of *iab-2*$^+$ can be indirectly inferred by comparing tracheal and cuticular patterns in AB2 of genotypes *d*, *e* and *f* (Fig. 6), which involve loss, weakened dominance and dominance, respectively, of *iab-2*$^+$. Continuity of DLT is seen in both *e* and *f*, suppression of KO occurs only in *f*, and VSB become progressively more abdomen-like in proceeding from *d* to *e* to *f*. These effects are not necessarily solely due to the *iab-2*$^+$ gene since it is possible that *iab-3*$^+$ is weakly derepressed in AB2 of the larva. Unfortunately, definitive genotypes are not available that would resolve *iab-2*$^+$ and *iab-3*$^+$ larval functions.

A comparison of larvae of genotypes *a* and *d* (Fig. 6) shows that the latter retain the wild-type cuticular pattern in AB8 (except for thoracic-type VSB) and have DLT continuity between AB7 (or AB6) and AB8. These effects are most simply accounted for by assuming that in contrast to *Df-P9*, *Df-Ubx*^{109} still retains an *iab-8*$^+$ gene, whose product, S_x, effects LAB7 → LAB8 or LMS → LAB8. The ability of genotype *d* to restore a tracheal trunk in the region from AB6 to AB8 suggests that *Ubx*^{109} retains additional *iab* loci to the right of *iab-3*, besides *iab-8*.

The above findings point to an antero-posterior gradient within the organism in BX-C gene action. A regulatory gene(s) might therefore be expected to exist which, when activated, would derepress or activate BX-C genes in all segments of the body. The properties of Polycomb (*Pc*), a mutant found by P. H. Lewis[15], and of a more extreme allele, *Pc*^{3}, suggest that *Pc*$^+$ (locus 3-47.1)[16] is such a gene. Thus, hemizygotes and homozygotes for *Pc* or *Pc*^{3} have recently been found to have their thoracic and first seven abdominal segments partially transformed towards LAB8. Such animals, which die as late embryos, also have reduced head structures, including poorly developed and incompletely sclerotised MH. As the doses of BX-C are increased from two to three to four, the transformation towards LAB8 becomes increasingly more extreme with many of the abdominal segments containing rudimentary posterior-type spiracles. The LAB8 transformation is not observed unless at least one dose of BX-C is present; that is, *Pc*^{3} *Df-P9* homozygotes closely resemble *Df-P9* homozygotes (Fig. 5) except that the former have reduced head structures and incompletely sclerotised CP in AB8 (suggesting that the latter structures are indeed rudiments of MH). A dependence of BX-C phenotypic effects on BX-C dosage is seen also in *Pc*^{3} heterozygotes: for example, *Cbx*-like effects occur in *Pc*^{3}/+ animals (with two doses of BX-C) and become increasingly more extreme with three and four doses of BX-C. Gene dosage studies of R. Denell (personal communication) indicate that *Pc* represents the inactivated state of the gene. Therefore, *Pc*$^+$ in all likelihood is coding for a repressor of BX-C.

Regulatory rules

The BX-C genes are assumed to control the organism's thoracic and abdominal segmentation pattern by producing substances which in turn regulate other genes that actually determine segmental structure and function. Regulation of the BX-C genes, themselves, seems to be governed by the following rules: (1) state of repression or derepression of a given gene is controlled (in at least four instances) by a *cis*-regulatory element; (2) the genes tend to be individually, rather than coor-

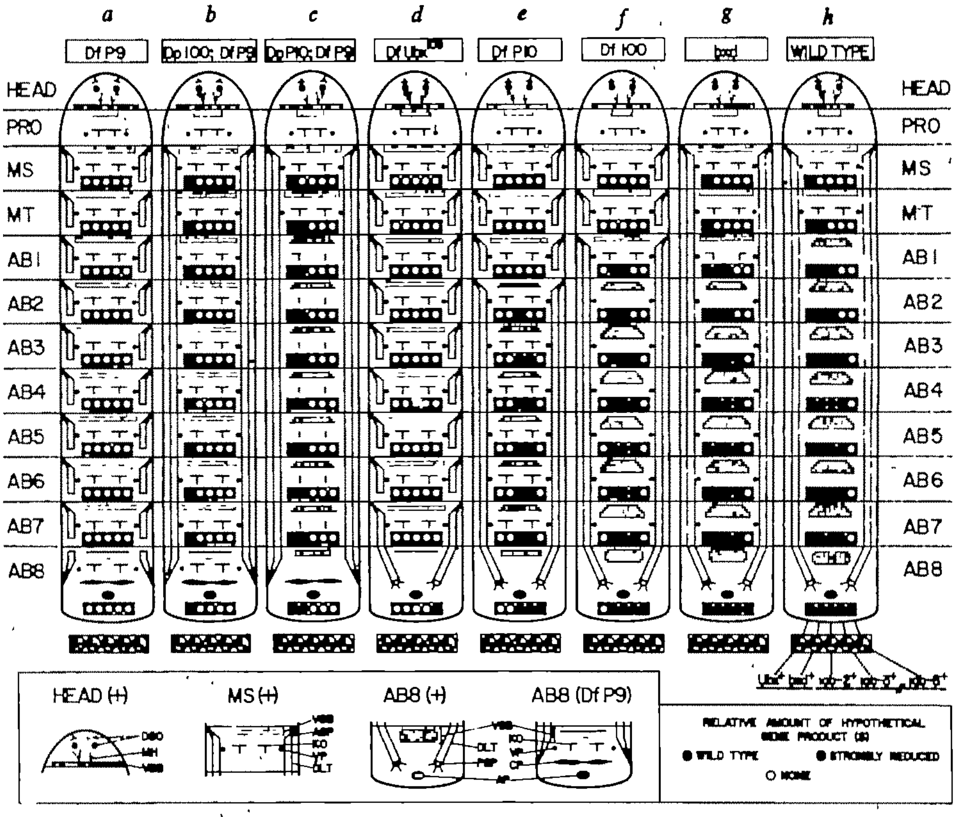

Fig. 6 Stylised phenotypes detectable in first instar larvae or mature embryos. See Fig. 1(*a*) for legend, Fig. 2 for description of rearrangements and Table 1 for roles of postulated BX-C substances (S). All drawings based on whole mounts of animals examined with phase contrast microscopy. Mounting fluid: 9 parts lactic acid to 1 part 95% ethanol for revealing cuticular structures; and Zeiss W15 for tracheal structures. *a*, *Df-P9* homozygote (see also Fig. 5*a* and *c*). *b*, *Df-P9* homozygote containing one dose of *Dp-100*, abbreviated symbol for the duplication derived from *Tp(3)bxd*^{100} (Fig. 2). Note identity to *a* except for presence of DLT *c*, *Df-P9* homozygote containing one dose of *Dp-P10*, abbreviated symbol for the duplication derived from *T(2; 3)P10* (Fig. 2). Note suppression of VP and partial suppression of KO in segments AB1 to AB7. Although VP and KO appear to be absent in AB8, they may be obscured by CP and other structures in that segment. *d*, *Df-Ubx*^{109}/*Df-P9*. Note DLT between segments AB7 and AB8, and presence of PSP in AB8; and absence of CP in AB8; occasionally DLT also forms between AB6 and AB7. *e*, *Df-P10/Df-P9*. *Df-P10* is an abbreviated symbol for the deficiency derived from *T(2; 3)P10* (Fig. 2). DLT restored in AB2 through AB8. The pair of separate sections of that trunk in AB1 frequently lie in AB2, parallel to DLT in that segment. Note partial restoration of abdominal-type VSB, weakly developed in AB2 and moderately developed in AB3 through AB8. *f*, *Df-100/Df-P9*. *Df-100* is an abbreviated symbol for the deficiency derived from *Tp(3)bxd*^{100} and has moderately weakened dominance of *bxd*$^{+}$ as a position effect of the 89E breakage point. Note retention of DLT and failure of suppression of VP in AB1 through AB7 (with VP status in AB8 uncertain) and full restoration of abdominal type of VSB in all segments from AB2 through AB8. *g*, *bxd/Df-P9*. Although not deleted for any known genes of BX-C this genotype is depicted since it has effects on several larval (as well as adult) cuticular structures. Note failure of suppression of VP on AB1 through AB7; thoracic-type VSB on AB1; and only partial suppression of KO on AB1. *h*, Wild-type homozygote or hemizygote (the latter having possibly somewhat narrower VSB on AB segments than the former) (see Fig. 5*b* and *d*). All individuals of genotype *a* through *f* die in the late embryonic or early first instar stage. ASP are lacking in first instar larvae according to Bodenstein[14]. Separate sections of DLT send out a branch to the cuticle but no anterior spiracles as such have been resolved. A number of larval phenotypic expressions are not readily explained unless it is assumed that there is weak derepression of *Ubx*$^{+}$ (to establish continuity of DLT between MS and MT), *bxd*$^{+}$ (to make very slightly larger VSB teeth in MT than MS) and *iab-2*$^{+}$ (to suppress KO in AB1) in the segment anterior to that in which each is fully derepressed. Whether *iab-3*$^{+}$ or *iab-8*$^{+}$ are also similarly weakly derepressed is uncertain.

dinately, derepressed; (3) BX-C is under negative control maintained by a major regulatory gene, *Pc*$^{+}$ (along with perhaps other regulatory genes[7,17]); (4) a gene derepressed in one segment is derepressed in all segments posterior thereto; (5) the more posterior the segment (starting with MT) the greater the number of BX-C genes that are in the derepressed state; and (6) the more proximal the locus of a gene in the complex the more likely it is that that gene is in a derepressed state. Rules (5) and (6) suggest that two types of gradient are involved in BX-C regulation: an antero-posterior gradient within the organism in repressor concentration and a proximo-distal gradient along the chromosome in relative affinity for repressor of *cis*-regulatory elements. Presumably in MT repressor concentration is so high that the only genes derepressed are those whose *cis*-regulatory elements have relatively low repressor affinities, while in AB8 repressor concentration is so low that all of the genes of the complex escape repression.

During ontogeny the above rules presumably result in each segment having a specific array of BX-C substances, at the right time, at the right place. In AB8, for example, the rules predict a minimum of eight such substances, the number of BX-C genes being very likely to exceed the eight identified thus far. Whether such a multiplicity of substances act in a hierarchical or a compartmentalised manner[18,19] to establish the final pattern or level of development of a segment may well be accessible to analysis at the cellular level.

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articles

A new class of radio star

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Radio observations of point sources are presented which may be the stellar remains of supernovae. In particular 1909+048 is described, a source which has also been detected in the optical and X-ray ranges.

THE stellar remnants of the Crab and Vela supernovae are detectable over a wide frequency range (radio through optical to X rays) as pulsars showing the short period (<1 s), presumably caused by fast rotation, in all observations. Although it has commonly been assumed that the stellar remnants of all supernovae form pulsars, there have been indications that stellar remnants may sometimes be detectable as non-pulsing radio sources. In particular: (1) Clark *et al.*[1] questioned whether Cir X-1 (with its associated non-pulsing radio source 1516−569) might be the stellar remnant of the supernova which formed the nearby radio shell SNR (supernova remnant) G321.9−0.3; (2) the point radio source 2048+312 (CL4), in the direction of the Cygnus Loop, may be associated[2] with the SNR; (3) an unusual point source 0125+628 at the centre of the SNR G127.1+0.5 may be[3] the stellar remnant of this supernova. This source has a flat spectrum[4] between 2.7 and 15 GHz, while its angular size is <1 m arc s and may be much less[5].

We have recently investigated the point radio source 1909+048 in W50 and have concluded that it certainly represents a distinct new type of radio source. We discuss here this object and some other possible examples for which we have preliminary measurements.

1909+048—the stellar remnant of the SNR W50?

The extended radio source W50 (G39.7−2.0) was recognised as an SNR by Holden and Caswell[6]. More recent data[7,8] confirm that it has a shell structure and reveal a point source near its centre; a small-diameter X-ray source (W50X) has also been reported near this direction[9,10]. We have used the Cambridge 5-km radio telescope at 2.7 and 15 GHz to study this point

Table 1 Candidate positions and their possible optical counterparts

Source	Name	Radio position RA(1950.0 coordinates) h min s	Radio position dec ° ′ ″	Mean date of radio position	Possible optical counterpart RA(1950.0 coordinates) h min s	Possible optical counterpart dec ° ′ ″	Notes
0125+628		01 25 08.366 ±0.011	62 50 59.02 ±0.03	1977 May	01 25 08.4	62 50 59.1	Optical position fro D. Wills (personal communication)*
0503+466		05 03 40.935 ±0.005	46 41 46.56 ±0.03	1977 April	—	—	No optical candidat visible on Palomar Sky Survey Prints.
1347−617		13 47 04.1 ±0.3	−61 45 54.4 ±2	1977 July	—	—	Position from Fleu Synthesis Telescop
1516−569	Cir X-1	15 16 48.45 ±0.2	−56 59 11 ±2		15 16 48.51 ±0.06	−56 59 12.7 ±0.5	Positions[17]
1849+005		18 49 13.597 ±0.012	00 31 54 ±2	1977 October	Crowded field		Declination from Fleurs Synthesis Telescope[16]
1909+048	W50X	19 09 21.273 ±0.005	04 53 53.1 ±0.5	1977 March	19 09 21.32 ±0.05	04 53 53.8 ±0.4	See text
1910+052		19 10 26.315 ±0.003	05 12 51.9 ±0.3	1977 May	Blank field		Nothing visible o POSS prints with 20 arc s
2013+370		20 13 37.009 ±0.007	37 01 44.51 ±0.06	1977 March	Crowded field		
2048+312	CL4	20 48 47.380 ±0.002	31 16 10.95 ±0.04	1974–75	See ref. 18*		Radio position[2]

* See note added in proof.

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source, and have found that the angular size is extremely small, the mean spectrum is relatively flat, and that the flux density varies dramatically at high frequencies (Fig. 1). Since this paper was completed, Seaquist *et al.*[11] and Feldman *et al.*[12] have also reported observations of the radio variability, and have noted that the optical object is No. 433 in the list of Hα-emission objects by Stephenson and Sanduleak[13]. An accurate position has also been measured (Table 1). The unusual nature of the radio/X-ray source and its position near the centre of the SNR shell strongly suggest that it is the stellar remnant of the supernova. A stellar object is indeed visible on the Palomar Observatory Sky Survey prints which coincides with the radio source to within the limits of error. The optical field is a crowded one, but the star in question is the brightest in the vicinity of the radio source. The estimated magnitudes are $m = 12.4 \pm 0.5$ on the E print and $m = 16.7 \pm 0.5$ on the O print. Clearly, it will be important to obtain an optical spectrum of this star[14], and to investigate the X-ray properties in greater detail.

A comparison of the distances of the point source and the shell SNR is obviously desirable. In the case of the point source, an HI absorption measurement is needed, but for the SNR we have estimated the distance from the Σ-D relation of Caswell and Lerche[15]. In reaching this estimate we first reassessed the radio data and adopted a spectral index $\alpha = 0.45$ (where $S \propto \nu^{-\alpha}$), S(408 MHz) = 65 Jy and elliptical major and minor axes of 81 and 45 arc min respectively. The corresponding surface brightness at 408 MHz, $\Sigma = 2.72 \times 10^{-21}$ W m^{-2} Hz^{-1} sr^{-1}, yields a distance of 3.3 kpc.

There is also a second point source (1910+052) within the shell of W50 which seems to be of the same type. It is included in Tables 1 and 2, and is discussed later.

Properties of the new class of source

Tables 1 and 2 list the candidates already mentioned, together with some further possibilities. Table 1 gives positions, as measured with the 5-km telescope except in the cases of 1347−617 and 1516−569, and also positions of optical objects which may be associated. In the case of 1909+048, the optical position has been newly measured from the Palomar Observatory Sky Survey prints, with respect to stars from the Smithsonian Astrophysical Observatry catalogue. Table 2 lists the flux densities at 1.4 GHz, angular diameters (from the 5-km observations if not otherwise noted), spectral indices, variability (see Fig. 1), estimated distances of the SNRs (derived from the Σ-D relationship of Caswell and Lerche[15], except for G74.9+1.2), and associations with SNRs.

From a consideration of the four candidates, 0125+628, 1516−569, 1909+048 and 2048+312, we suggest the following characteristic properties of this class of source: (1) small diameter; (2) variability at high frequencies; (3) flat spectrum at high frequencies; (4) association with an SNR. In general a position near the centre is expected unless the object has a large space velocity. If the lifetime of these compact radio emitters is greater than that of the shell remnants, however, it is evident that there will be many without associated shells, as in the case of the radio pulsars. (5) Possible X-ray and/or optical counterparts.

In addition to the four sources already considered, Tables 1 and 2 list the properties of five other sources which have been found near SNRs and which we also regard as possible members of the new class. Some additional details of the sources, not previously mentioned, are as follows.

0125+628. From a small number of observations, Salter *et al.*[4] detected no variations at 5 GHz, but it is clear from Fig. 1 that the flux density at 15.4 GHz is varying on a timescale of a month, and perhaps much faster, although the significance of the more rapid variations is not large. Figure 1 suggests that $\alpha_{2.7}^{15} \approx -0.2$ in 1977 May, and the flux values at 2.7, 8.1 and 10.6 GHz quoted by Shaffer *et al.*[5] also confirm the flatness of the spectrum above about 2 GHz. There is a cut-off at lower frequencies.

0503+466. Point source in HB9, with S(2.7 GHz) = 590 mJy (ref. 21). It is absent from the 4C records but has been detected in the 6C survey[22] with S(150 MHz) = 740 ± 250 mJy in 1976 May (Masson, personal communication). Its spectrum is therefore very flat.

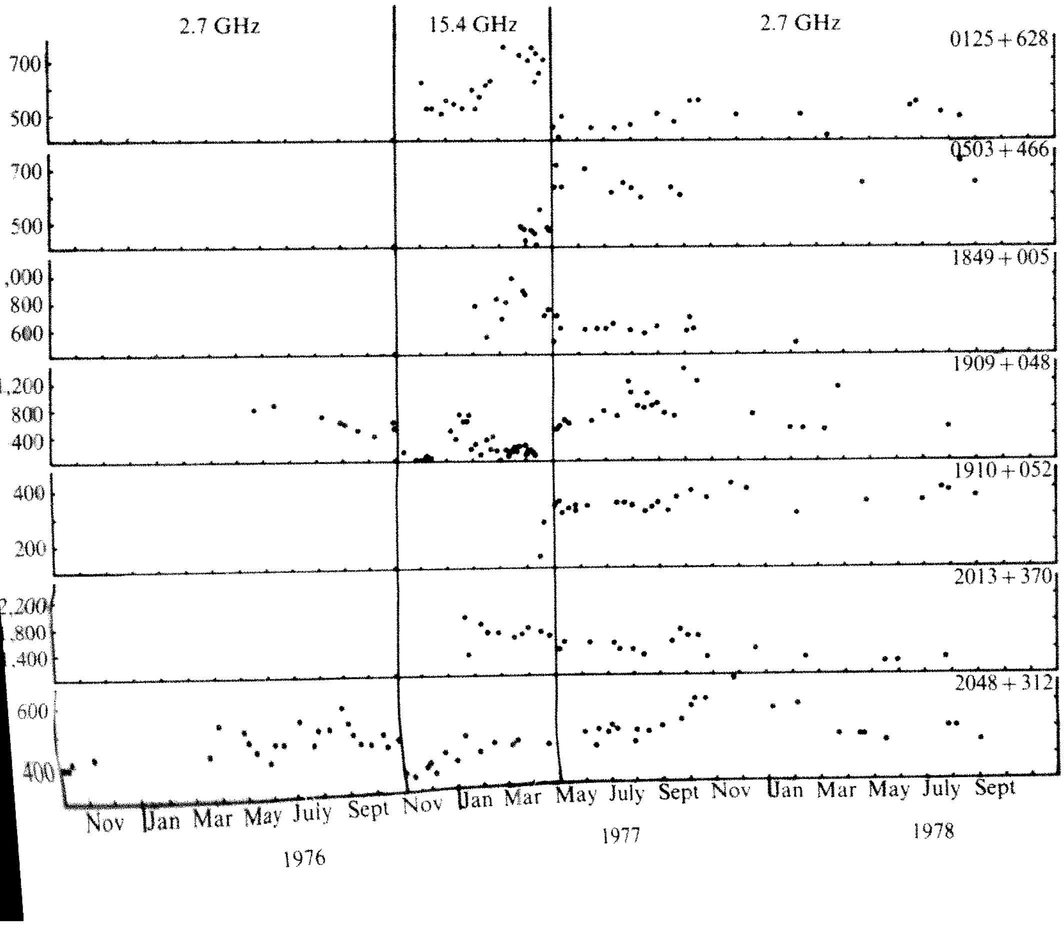

Fig. 1 Flux densities as measured with the 5-km telescope at 2.7 GHz (October 1975–October 1976, and May 1977–September 1978) and 15.4 GHz (November 1976–April 1977). Linearly polarised feeds were used with the E-vector in position angle 90°. The calibration assumed flux densities S(2.7 GHz) = 13.0 Jy for 3C147, and S(2.7 GHz) = 10.3 Jy, S(15.4 GHz) = 3.69 Jy for 3C286. The main contributions to errors are from receiver noise (~3 mJy at 2.7 GHz, and 20 mJy at 15.4 GHz) and tropospheric variations (<3% at 2.7 GHz, and <5% at 15.4 GHz).

Table 2 Candidate flux densities and other data

Source	S(1.4 GHz) mJy	Angular diameter 10^{-3} arc s	Spectral Index	Variable	SNR distance estimate (kpc)	Nearby SNR	Refs
0125+628	380	≈0.5	$\alpha_{2.7}^{15} = -0.2$	Yes	5.4	At centre of G127.1+0.5 (R5)	3, 5, 19, see text
0503+466	≈600	<150±130	$\alpha_{2.7}^{15} \approx 0.2$	Yes	1.8	At edge of G160.5+2.8 (HB9)	See text
1347−617	230	>15 000	$\alpha_{0.4}^{1.4} = 0.71$	?	8.1	Near centre of G309.8+0.0	See text
1516−569 (Cir X-1)	380	—	$\alpha_{0.4}^{5} \approx 0.25$	Yes	8	Near G321.9−0.3	1, 17, 20
1849+005	≈800	<130±130	$\alpha_{1.4}^{15} \approx 0.1$	Yes	9.4	Near G33.6+0.1	See text
1909+048 (W50X)	≈650	<150±100	$\alpha_{0.4}^{5} \approx 0.1$	Yes	3.3	Near centre of G39.7−2.0 (W50	See text
1910+052	≈600	<320±210	$\alpha_{0.4}^{15} \approx 0.3$	Yes	3.3	Within G39.7−2.0 (W50)	See text
2013+370	1150	≈0.5	$\alpha_{2.7}^{15} \approx 0$	Yes	>12	Near G74.9+1.2	See text; 19
2048+312 (CL4)	380	≈1	$\alpha_{1.4}^{15} \approx 0.21$	Yes	1.2	Within G74.0−8.5 (Cyg Loop)	2, 19

1347−617. This source is listed tentatively, on the grounds of its position very near the centre of the SNR G309.8+0.0. It contrasts with the other sources by apparently having a steeper spectrum, but flux variability might be responsible.

1849+005. This source was first noted by Caswell *et al.*[23] while mapping the SNR G33.6+0.1, and attention was also drawn to it as a possibly unusual galactic source by Caswell *et al.*[24]. The spectral index quoted in Table 2 is based on a 5-km value of S(15 GHz) = 700 mJy.

1909+048. Figure 1 shows that this is highly variable, especially at 15 GHz. At epoch 1976.9, for example, S(15 GHz) < 20 mJy but, within two months, S(15 GHz) was 740 mJy.

1910+052. This second point source within the shell of W50 was first detected with S(408 MHz) = 780 mJy in 1974. Like 1909+048, it has a small angular size, a flat spectrum and is almost certainly variable at 2.7 GHz (Fig. 1). There were two measurements at 15.4 GHz; these indicated a stronger variability but this requires confirmation. The flux density was also measured at Parkes, New South Wales as S(5 GHz) = 430 mJy in May 1974 and S(15 GHz) = 230 mJy in 1977 August. The occurrence of this second point source within a single SNR shell may be a chance superposition. It is clear that more statistical data on the spatial density of such point sources are required before we can evaluate this possibility.

2013+370. This region has been studied by Duin *et al.*[25] and Shaffer *et al.*[5] The point source lies about 7 arc min outside the SNR boundary so, if the association is real, a high velocity of ejection (>5,000 km s^{-1}) would be necessary to account for the separation. HI absorption measurements[26] place similar lower limits (of ~12 kpc) on the distance of both objects. Whereas the extended source is almost certainly a galactic SNR, present evidence does not rule out an extragalactic nature for the point source. The spectrum is, nevertheless, unusual by any standards, with a low frequency cut-off at the very high frequency of 7.5 GHz.

Discussion

Most of the sources investigated probably represent an important new class of radio star. The relationship of these sources to the other galactic X-ray sources and to pulsars is not yet clear although, if either 1909+048 or 1516−569 is indeed a member of the new class, then the other galactic X-ray binary systems (with or without detectable radio emission) may, in some cases at least, also be the stellar remnants of supernovae, perhaps having outlived their SNR shells.

It has been proposed[27] that all supernovae are formed in binary systems; this would be consistent with the X-ray binary stars originating in supernovae, and may indicate that the radio pulsars are generally confined to those binary systems which have been disrupted by the supernova explosion (in particular, the two pulsars definitely associated with SNRs are not binary systems). If sources of the type discussed here are associated with binary systems not disrupted by the explosion, one may expect periodicities in the radio variations. Webster & Ryle[2], in fact, suggested that the flux variations of 2048+312 are quasi-periodic with a period of ~60 d; we have carried out a power-spectrum analysis on all the available observations. This did not confirm the suggestion but, because of the irregularity of the observations, we cannot reject it either.

A major problem is that the radio properties of the new class are superficially similar to those of some extragalactic sources, the BL Lac objects, and it is indeed possible that some of our suggested examples of stellar remnants may be misidentified. Nevertheless, there can be no doubt that both types of source exist—some, such as the radio counterpart of Cir X-1, being definitely galactic, and others such as the BL Lac objects showing large redshifts, being definitely extragalactic.

We thank Dr B. Elsmore for providing the 5-km radio positions, C. R. Masson for the 150-MHz flux density of 0503+466, and Dr D. Wills for the optical position of the candidate identification for 0125+628, also Drs J. E. Baldwin, P. A. G. Scheuer and A. S. Webster for valuable comments.

Note added in proof—A spectrum[28] of the possible optical counterpart of 0125+628 reveals a faint galaxy. Thus if the radio–optical association is valid, this radio source is not galactic and its coincidence with the SNR is by chance. A red object[29] of 21 mag coincides with the radio source.

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K–Ar age of the late Pleistocene eruption of Toba, north Sumatra

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The late Pleistocene eruption of Toba is the largest magnitude explosive eruption documented from the Quaternary. K–Ar dating of the uppermost unit of the Toba Tuff gives an age of ~75,000 yr. A chemically and petrographically equivalent ash layer in deep-sea cores helps calibrate the Stage 4–5 boundary of the standard oxygen isotope stratigraphy. A similar ash in Malaya that overlies finds of Tampan Palaeolithic tools indicates that they are older than 75,000 yr.

A WIDESPREAD volcanic ash layer has been found in piston cores taken in the north-east Indian Ocean and the Bay of Bengal, where it occurs in the upper 12 m of sediment. This ash layer has been correlated from core to core on the basis of mineralogy[1]. A study of the area distribution, thickness, grain size and volume of the deep-sea ash layer has shown that the ash originated in an eruption an order of magnitude larger than any other documented for the Quaternary[2] and indicates a source of the ash in an eruption of Toba, north Sumatra[1,2]. Toba caldera, whose dimensions are 100 × 30 km, is surrounded by rhyolitic tuffs (Fig. 1) which occupy an area of 20,000–30,000 km^2 and have a thickness of several hundred metres (refs 3, 4). We report here on the radiometric dating of the Toba Tuff and the correlation of this eruptive.

Several samples of Toba tuffs have been dated. A sample from the base of the tuff series has been dated by the K–Ar method at 1.9 Myr (ref. 5). Three other samples have been dated by the fission-track method: samples of 'old tuff' and 'upper tuff' from the Prapat area gave 1.2 and 0.1 Myr respectively. A third sample of 'welded tuff' from the area of Si Gura Gura gave an age of 0.1 Myr (ref. 6).

A K–Ar age of $75 \pm 12 \times 10^3$ yr on biotite has been reported[7] from a Toba welded tuff sample Id 680. We report here the data on the K–Ar dating and on the chemical and mineraological composition of sample Id 680 from Si Gura Gura and a sample of pumice tuff (72–0–60) from the Prapat area. We have also analysed chemically and mineralogically an ash layer which occurs in 15 deep-sea cores to the west and north-west of Toba (Fig. 1). We have established the position of the ash layer in one of the cores in the standard oxygen isotope stratigraphy, confirmed by two biostratigraphic data, to the extinctions of the radiolarian *Stylatractus universus* and coccolith *Pseudoemiliania lacunosa*. We have also analysed several samples of ash from the Kota Tampan area in west Malaya.

Toba tuffs

The welded tuff sample Id 680 (from the collection of Dr M. T. Zen) came from Si Gura Gura (Fig. 1), along the Asahan River, and was taken at a depth of 55 m.

The tuff consists of about 47% crystal fragments embedded in a matrix of fine crystalline material and volcanic glass. The composition of heavy minerals is given in Table 1. Only a few phenocrysts of allanite, reported by Westerveld[8], have been identified in the studied sample. Opaque minerals are rare, and are mostly developed as secondary products on grain borders or within xenoliths. Xenoliths are abundant in the form of schist. The light mineral fraction was analysed using the methods described by Hayes and Klugman[9] and the results plotted in Fig. 2. The volcanic glass has a refractive index of 1.496–1.500. Wet chemical analysis has been done on both the whole rock and pure glass shard fraction (Table 2). K–Ar dating was done on biotite and gave an age of $74.9 \pm 12 \times 10^3$ yr (Table 3).

Table 1 Composition of the heavy mineral fraction (%) in the Toba welded tuff and in the ash layer from Tampan and deep-sea cores (size fraction: 0.25–0.062 mm)

	Biotite	Hornblende	Hyperstene	Epidote	Muscovite	Tourmaline	Total (%)
Toba							
(Id 680)	86.0	12.0	2.0	—	—	—	100
RC14-37	84.0	16.0	—	—	—	—	100
RC14-38	72.0	27.5	0.4	—	0.1	—	100
RC17-145	79.0	20.0	1.0	—	—	—	100
Tampan							
6312	74.0	10.0	—	4.0	3.0	9.0	100
6313	85.0	3.0	0.3	0.7	5.3	5.7	100
6315	91.0	6.0	—	—	3.0	—	100
6317	86.0	7.0	—	—	3.6	3.4	100
		Pyrogenic			Detrital		

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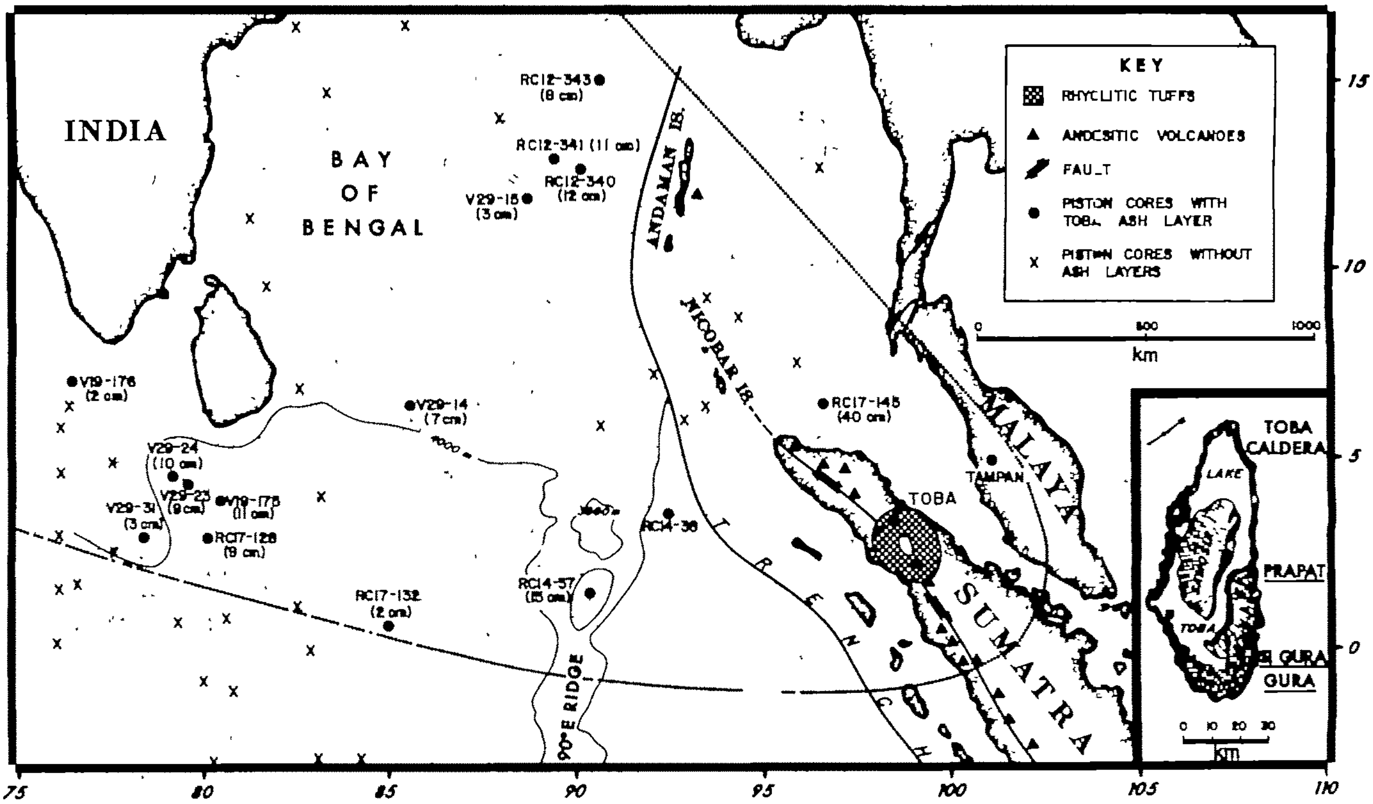


Fig. 1 Map showing the distribution of Toba tuffs on Sumatra and the equivalent ash layer in deep-sea cores and on Malaya (after refs 1 and 2). The inset shows the location of Si Gura Gura and Prapat to the east of Toba caldera, where the analysed samples of tuffs were collected.

Table 2 Chemical composition of the Toba welded tuff and the ash layers in deep-sea core and on Malaya

	1	2	3	4
SiO_2	70.0	72.2	71.8	72.6
TiO_2	0.37	0.11	0.14	0.16
Al_2O_3	13.9	12.9	13.1	13.6
Fe_2O_3	2.0	0.3	0.15	0.75
FeO	0.9	0.7	0.85	0.05
MnO	0.06	0.06	0.07	0.05
MgO	0.8	0.35	0.25	0.1
CaO	1.8	2.2	1.65	1.0
Na_2O	3.4	2.8	3.7	2.5
K_2O	5.2	5.1	5.1	4.6
P_2O_5	0.06	0.03	0.05	0.06
H_2O	1.4	3.1	3.0	4.2
Total	99.89	99.85	99.86	99.67

Analyst M. Weibel.

1. TOBA (Si Gura Gura, Id 680); whole rock.
2. TOBA (Si Gura Gura, Id 680); glass shard fraction.
3. Deep-sea core RC14-37; ash layer at 100–115 cm, glass shard fraction.
4. Kota Tampan, Malaya; volcanic ash (UM6315); glass shard fraction.

The pumice tuff (72-0-60) sample was collected by J. D. Obradovich at Prapat (Fig. 1), where in 1972 an excavation above the lake exposed some 10 m of pumice tuff. The pumice fragments (5–10 cm) are predominantly glass with phenocrysts (2–4 mm) of quartz, sanidine, black biotite and brown hornblende; zircon was also recovered. No xenoliths are present.

K-Ar dating was done on sanidine and gave an age of $73.5 \pm 3 \times 10^3$ yr (Table 3). Because the radiogenic argon content for the analysis on sanidine is so much greater than that of the biotite analysis this result is considered to be the most reliable.

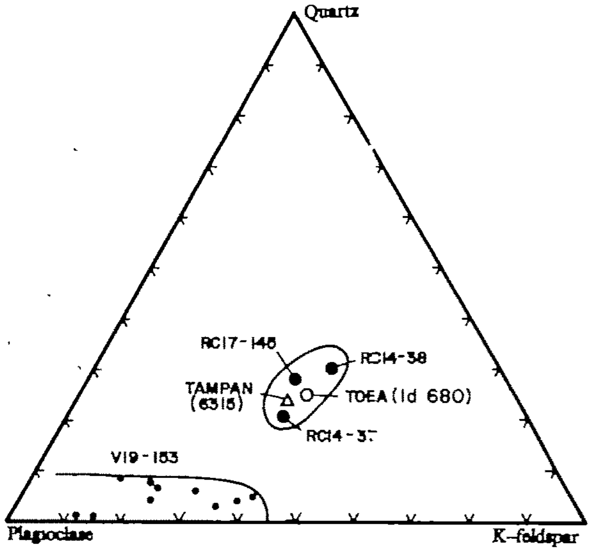
Toba ash layer in deep-sea cores

Fifteen deep-sea cores (Fig. 1) contain a single ash layer. Glass shards from the ash layer in all cores have a refractive index of 1.496–1.500, similar to that of the Toba tuff. Mineralogical composition of the deep-sea ash layer is also similar to the Toba sample (Table 1, Fig. 2). Chemical composition of the glass shard fraction from the ash in core RC14-37 is given in Table 2.

RC14-37 was taken from the top of the 90° E ridge. The ash layer, graded from the bottom upwards, occurs at 100–115 cm (Fig. 3). The core is composed of about 85% $CaCO_3$, and shows very little variation throughout the sediment.

Oxygen isotope measurements were made in *Globigerinoides*

Fig. 2 Correlation of upper Toba tuff with ash layer from Tampan, Malaya and deep-sea cores RC14-37, RC14-38 and RC17-145, based on light mineral composition.



sacculifer with a view to establishing the position of the ash layer in the standard oxygen isotope stratigraphy. The measurements (Fig. 3) indicate that the core extends to Stage 13 at about 0.5 Myr. This is confirmed by the observation that Stage 12 is bracketed by the extinction of *P. lacunosa* below[11], and *S. universus* above[12]. The Toba ash layer is found at about the Stage 5–4 boundary (Fig. 3). The age of the Stage 5–4 boundary is generally agreed to be ~75,000 yr (ref. 13).

The recognition of the Toba ash in core RC14-37 and its K–Ar age of $73.5 \pm 3 \times 10^3$ yr allows an independent assessment of the age of the Stage 4–5 boundary. As the ages of the stage boundaries given by Shackleton and Opdyke[13] are estimated on the basis of a uniform sedimentation rate in core V28-238 calibrated by the presence of the Brunhes–Matuyama magnetic epoch boundary, age 700,000 yr, our results confirm that this assumption is valid.

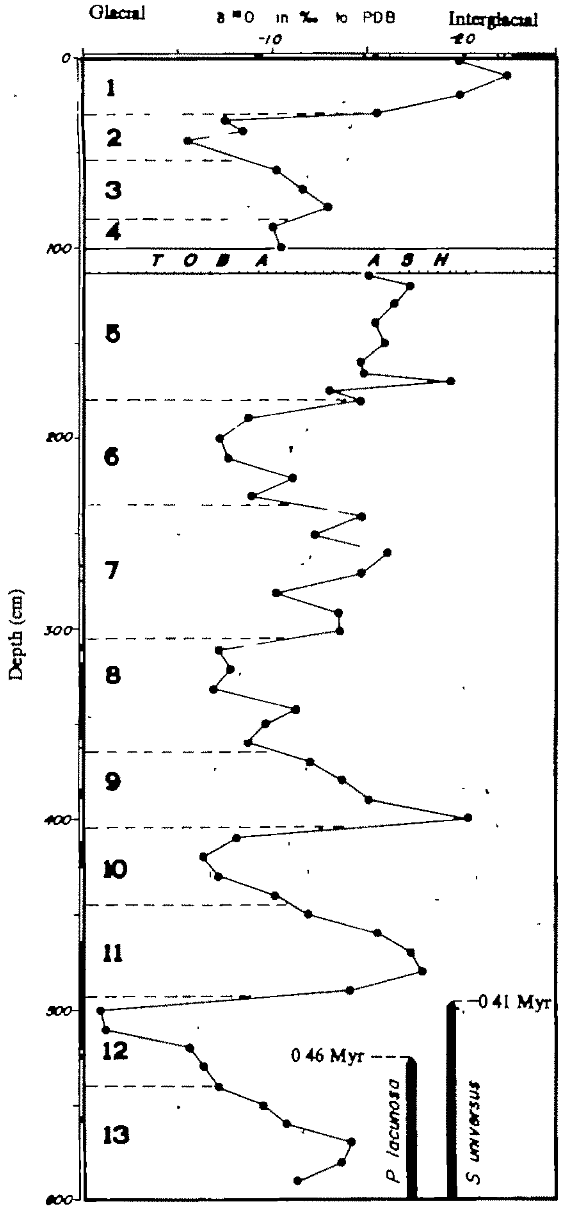


Fig. 3 Core RC14-37. Oxygen isotope curve, and coccolith *P. lacunosa* and radiolarian *S. universus* extinction levels (after refs 11 and 12). Toba ash occurs at about the $\delta^{18}O$ Stage 4–5 boundary.

Volcanic ash from Kota Tampan

Volcanic ash, up to 3 m thick was first reported from the Kota Tampan area in west Malaya by Scrivenor[14] and suggested by van Bemmelen[4] to have originated in the Toba eruption. This ash has received much attention, particularly because it covers gravels from an ancient river terrace in which Palaeolithic tools have been found[15] (Fig. 4). The tools have been studied archaeologically by Collings[15], Sieveking[16], and more recently by Harrison[17], who questions the validity of the Tampan artefacts.

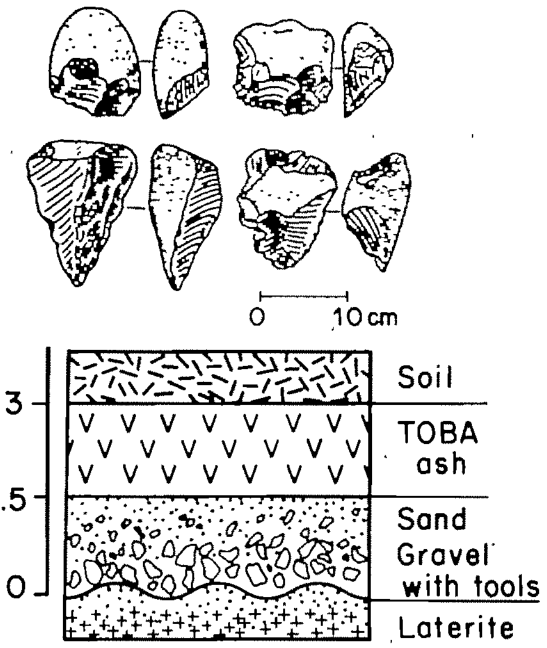


Fig. 4 Tampan Palaeolithic tools and their stratigraphic position (after ref. 15)

An age of about 35,000 yr for the Tampan Palaeolithic tools, attributed by Harrison, is based on ^{14}C dating of wood reported by Stauffer[18]. Three samples of wood found under the ash have been dated and gave the following ages: 33,250 ± 1,800, 36,500 ± 2,500, and greater than 39,000 yr BP. One sample of wood found above the ash layer gave an age of 1,145 ± 90 yr BP (ref. 18). In view of our results, however, it seems likely that the two samples that yielded apparently finite ^{14}C ages were contaminated by recent carbon (see Stuiver et al.[19] for an up to date discussion of contamination of samples by modern carbon).

Several samples of Tampan ash were provided by N. Haile. The following four samples have been analysed: UM6312 and 6313 (100°58′40″ E, 5°3′30″ N), UM6315 (100°57′50″ E, 5°3′25″ N) and UM6317 (100°57′30″ E, 4°49′10″ N). Volcanic glass shards from all samples have a refractive index similar to that of the Toba tuff (Id 680). Chemical composition of the

Fig. 5 Electron microprobe analysis of *a*, volcanic glass; and *b*, sanidine from upper Toba tuff and from volcanic ash on Malaya (analyst G. Izett). ●, Toba (72-0-60), ×, Malaya (UM 6313).

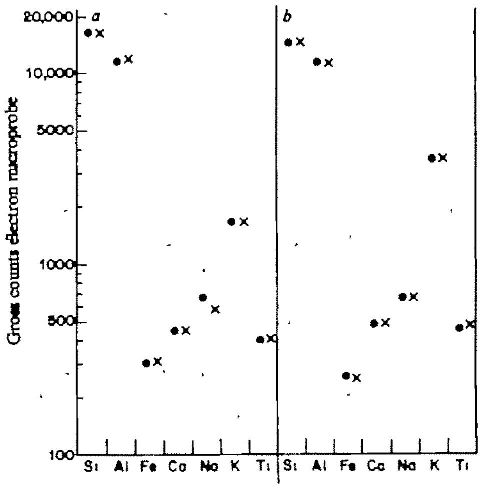


Table 3 Analytical data for Toba tuffs

Sample	Mineral	%K	mol ^{40}Ar* per g $\times 10^{-12}$	%Rac Ar	Age (10^3 yr)
Si Gura Gura	Biotite	6.17†	0.959	1.60	74.9 ± 12
Id 680§			0.634	0.56	
			av. 0.797		
72–0–60	Sanidine	9.88‡	1.259	13.[illegible]	73.5 ± 3
Prapat‖					

Decay Constants: ^{40}K/K = 1.167×10^{-4} atom/atom; $\lambda_\beta = 4.962 \times 10^{-10}$ yr^{-1}; $\lambda_{e+e'} = 0.581 \times 10^{-10}$ yr^{-1} (from Steiger and Jaeger[10]).

† Potassium by atomic absorption.

‡ Potassium by isotope dilution

§ Analyst A. A. Abdel-Monem.

‖ Analyst J D Obradovich.

samples is given in Table 1 and relative proportions of light minerals in Fig. 2.

The relative proportions of light minerals in sample UM6315 is similar to that of the Toba tuff and the deep-sea ash layer. The chemical composition of the pure glass shard fraction of sample UM6315 is given in Table 2. The three other samples have a higher quartz content, and contain a small per cent of tourmaline, suggesting contamination by residual sand.

Electron microprobe analysis for seven elements has been done on volcanic glass shards and sanidine from the Tampan sample UM6313 and Toba sample 72–0–60 and those data are plotted as gross counts in Fig. 5. The match for these seven elements is exceedingly good. The sanidine phenocrysts of both samples are notable in that they have an extremely high value for K_2O of 11.9% based on isotope dilution analysis of sample 72–0–60.

Volcanic ash layers in deep-sea cores near Sunda Strait

Several piston cores containing rhyolitic ash layers have been taken to the south-west of Sunda Strait[20], a few hundred kilometres from the rhyolitic tuff fields of south Sumatra and west Java. Core V19–153 contains 10 ash layers, whose age and chemical composition have been reported by Ninkovich[1].

The compositions of the light mineral fraction from 10 ash layers in core V19–153 are shown in Fig. 2, for comparison with the Toba products. It is obvious from these data that the source of the ash in Malaya and in core RC14–37 is in Toba and not the same as the source of the ash in the more southerly cores.

Conclusion

Sample Id680 from Si Gura Gura taken near the top of the Toba tuffs and the ash layer in deep-sea core RC14–37 have a similar chemical and mineralogical composition. K–Ar dating of the Toba tuff sample Id 680 from Si Gura Gura and sample 72–0–60 from Prapat, and oxygen isotope and biostratigraphic dating of the ash layer in core RC14–37 have provided a similar estimate of about 75,000 yr for the age of the eruption. Examination of core RC14–37 indicates that the 75,000 BP eruption was the only major explosive eruption of Toba in the past 0.5 Myr. Chemical and mineralogical composition of the thick Tampan ash suggests its source was also the 75,000 yr old Toba eruption. The K–Ar age of 73.5 ± 3 × 10³ yr obtained for sanidine from the eruption provides a valuable independent check on the age of the Stage 5–4 boundary in the standard oxygen isotope stratigraphy.

Toba tuff sample Id 680 was provided by Dr M. T. Zen, and ash samples from Malaya by Professor N. Haile. The manuscript was reviewed by F. McCoy and L. H. Burckle. The research was partly funded by NSF grant OCE-76-30690. The core curating facilities at LDGO are supported by the Office of Naval Research (contract N00014-75-C-0210-Scope E) and the NSF (contract OCE-76-18049).

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Asymmetric gene conversion at inserted segments on yeast mitochondrial DNA

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Different molecular weight forms of the protein product of the yeast mitochondrial gene var1 *are shown to arise by a process of asymmetric gene conversion. These different forms can be accounted for by two DNA segments, ~36 and 57 base pairs long, present in one allelic form of the* var1 *structural gene, which can be inserted independently and at different frequencies into other* var1 *alleles.*

THE mitochondrial genome of bakers' yeast *Saccharomyces cerevisiae* has been the subject of intensive genetic and biochemical investigation. The genome is apparently highly active in genetic recombination and many phenomenological aspects of the recombination, transmission and segregation of mitochondrial genes have now been well defined (see ref. 1 for a review). However, there is still little information available concerning the molecular mechanisms underlying these proces-

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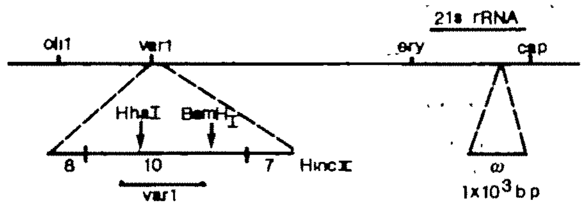

Fig. 1 Diagrammatic representation of the *cap–oli^r1* region of the yeast mitochondrial genome, showing the relative locations of the *var1* gene and the ω insertion, to markers for chloramphenicol resistance (*cap^r*), erythromycin resistance (*ery^r*) and oligomycin resistance (*oli^r1*). *Cap* and *ery* are located in the region encoding the 21S mitochondrial ribosomal RNA; this region is separated from *oli1* by a distance of 11×10^3 base pairs. The determinant ω, an insertion of 1,000 base pairs[10,11], is located within the 21S ribosomal DNA segment. Yeast strains whose mtDNA contains the insertion are termed ω^+; those whose mtDNA lacks it, ω^-.

ses. We know that in most cases, mitochondrial genetic markers are transmitted coordinately to the diploid progeny of crosses. Furthermore, markers tend to segregate rapidly during vegetative growth and for most alleles, reciprocal recombinant genotypes are recovered in equal numbers[2–6]. A well established exception to this last point involves a determinant located in the 21S ribosomal DNA region of the mitochondrial genome designated ω (refs 7, 8) (Fig. 1). Crosses having the configuration $\omega^+ \times \omega^-$ (termed heteropolar) produce grossly unequal numbers of genotypes recombinant for the genetic markers in the vicinity of the ribosomal DNA region[7–9]. Physical mapping studies of mitochondrial DNA (mtDNA) in yeast strains with ω^+ and ω^- alleles show that mtDNA from ω^+ strains has an insertion of 1,000 base pairs near the markers *cap* and *ery* (Fig. 1) that is absent from mtDNA of ω^- strains[10,11]. The major consequence of this difference seems to be a polarity of recombination in the ribosomal DNA region as a result of gene conversion events in the direction ω^- to ω^+. Thus, the 1,000-base pair insertion spreads in heteroplasmic cells among the population of mtDNA molecules. In homopolar crosses, where both parents have the same ω allele, either ω^- or ω^+, no polarity of recombination is observed[8].

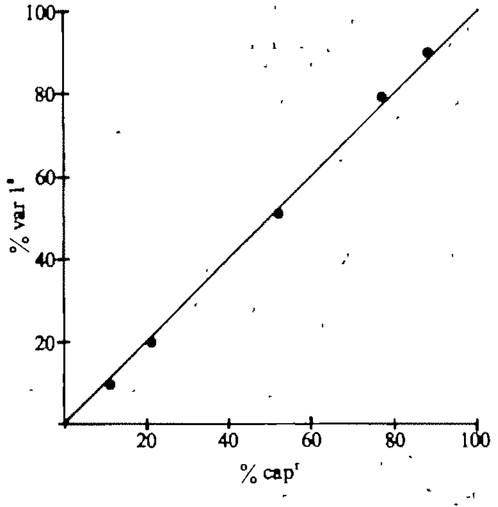

Fig. 2 Calibration curve for an assay of the ratio of *var1* alleles in mixed cell populations. Two diploid segregants of genotype *cap^r var1^S* (Mw 44,000) and *cap^s var1^F* (Mw 40,000) were obtained from the cross 5DSS (*cap^s var1^S*) × 195D (*cap^r var1^F*). These were grown to log phase at 28° C in YEP medium (1% yeast extract, 1% bactopeptone) containing 2% dextrose and were then mixed in various proportions. To determine the exact percentage of each cell type in the mixed cell population, an aliquot was plated on YEP medium containing 2% dextrose and then replica plated on to YEP medium containing 3% glycerol and 3 mg ml^{-1} chloramphenicol. Mitochondrial translation products of each mixed cell population were labelled *in vivo* with $^{35}SO_4^{2-}$ and analysed on SDS–polyacrylamide gels according to the procedure of Douglas and Butow[15]. The autoradiogram of the SDS–polyacrylamide gel was traced with a quick scan densitometer (Helena) and the gaussian curves representing var1 polypeptide were quantitated with a Dupont curve resolver.

Several other strain-specific differences involving various combinations of insertions and deletions in wild-type (ρ^+) mtDNA have been noted[11,12]. These seem to involve DNA segments from <100 to >1,000 base pairs. Fonty *et al.*[13] have reported that when two ρ^+ strains differing in *Hae*III and *Hpa*II restriction endonuclease fragment patterns are mated, a portion of the diploid progeny have one or more restriction fragments that are non-parental. It was suggested that these 'new' bands arise as a result of unequal crossing-over in dAT 'spacer' sequences known to comprise about 50% of the mtDNA in *S. cerevisiae*[14].

We have previously described a product of mitochondrial protein synthesis with novel properties[15]. When mitochondrially synthesised proteins from different ρ^+ yeast strains were analysed on SDS–polyacrylamide gels, most of the major mitochondrial translation products (three subunits of cytochrome oxidase, and the presumed apoprotein of cytochrome *b*) had identical mobilities in all strains. However, a protein termed var1, not previously associated with any mitochondrial function, was found to vary in electrophoretic mobility among the different ρ^+ strains analysed. Thus far, we have identified at least six strain-dependent forms of the var1 polypeptide ranging in molecular weight from 40,000 to 44,000 which are stable during vegetative growth[16]. This polymorphism was used as a genetic marker to show that the structural gene for the polypeptide is located on mtDNA between the markers *ery* and *oli1* (ref. 17) (Fig. 1). By a combination of petite deletion mapping, zygotic gene rescue[18], and restriction endonuclease analysis of ρ^+ and petite mtDNA samples, we have located the structural gene for the var1 polypeptide to a DNA fragment about 2×10^3 base pairs in length, beginning 9.7×10^3 base pairs from a unique *Sal*I site on mtDNA in ω^- strains and 10.7×10^3 base pairs from the same *Sal*I site in ω^+ strains[16] (R.D.V. *et al.*, unpublished data). An important conclusion drawn from our studies was that the variant forms are the result of sequence length differences in the *var1* structural gene itself.

In this report we present a detailed analysis of the origin of different forms of var1 polypeptide. The results provide a satisfying explanation for the apparent widespread polymorphism exhibited by this protein and may be of general significance concerning genetic exchanges in yeast mtDNA. The data demonstrate, at the molecular level, a process of asymmetric intragenic gene conversion which can occur in crosses at a frequency at least equal to that of the observed upper limit of recombination between unlinked mitochondrial markers[4]. The gene conversion events can result in either the conversion of one parental allele to the other, or the formation of new, non-parental forms of the *var1* gene. Although these events at *var1* are assayed differently from those at ω, there is a great similarity between the types of gene conversion exhibited at these two regions of the mitochondrial genome.

Assay for var1 polypeptides in mixed cell populations

In our previous genetic studies, the distribution in crosses of different forms of var1 polypeptide was studied by first isolating homoplasmic diploids and then analysing the individual diploid clones for their drug resistance and *var1* alleles[16,17]. Because the *var1* allele must be determined by analysis of mitochondrial translation products on SDS–polyacrylamide gels, the procedure becomes quite cumbersome for scoring *var1* transmission in a large number of crosses. Consequently, to facilitate the studies described here, we determined whether it was possible to measure quantitatively the relative amounts of different molecular weight forms of *var1* polypeptide in mixed cell populations.

To this end, we carried out a reconstruction experiment in which two isonuclear diploid clones, one of genotype *cap^r var1*

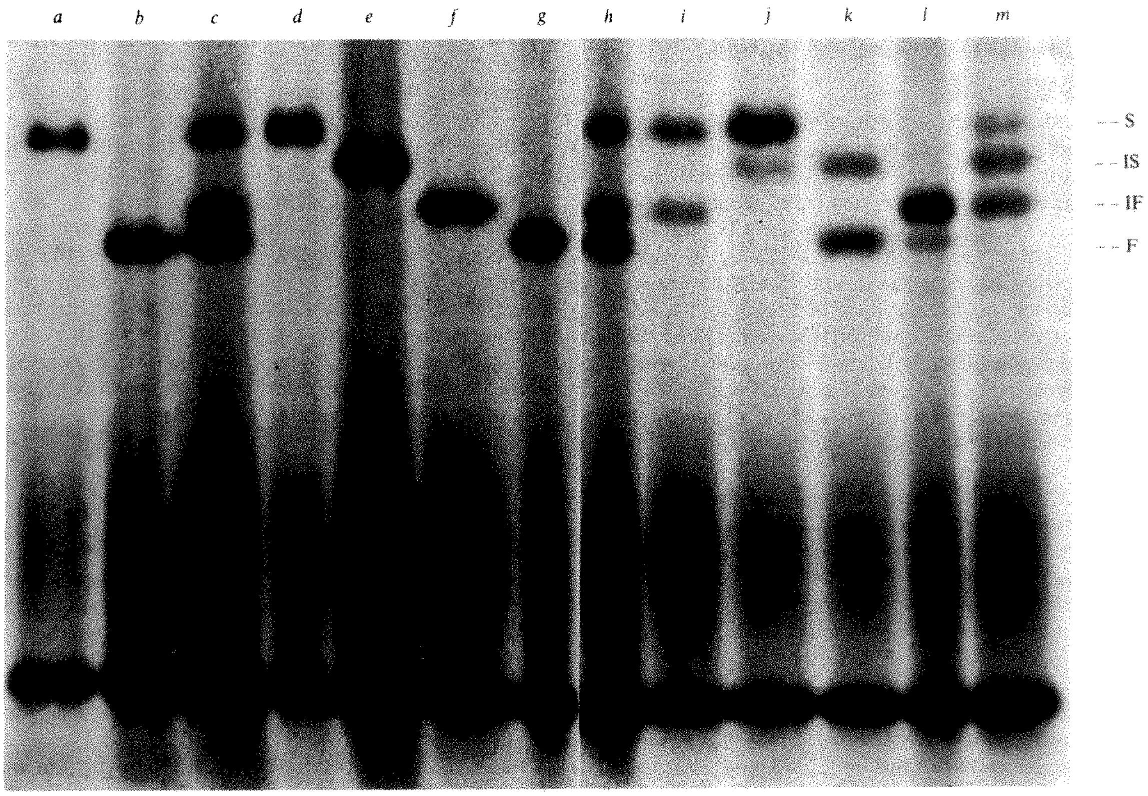

Fig. 3 Analysis of var1 polypeptide in crosses. Wild-type strain 5DSS (a *ura* ω^+ cap^s ery^r oli^s $var1^S$) and 195D (α *ade* '*ys* ω^+ cap^r ery^s oli^r $var1^F$) were mated. Random diploid[7] as well as homoplasmic diploid clones were obtained and their mitochondrial translation products analysed as indicated in Fig. 2. The *var1* alleles for the haploid strains 5DSS and 195D are shown in lanes *a* and *b*, respectively. Random diploid progeny from the cross are shown in lane *c*. Homoplasmic diploid segregants containing the four progeny *var1* types ($var1^S$, $var1^{IS}$, $var1^{IF}$, and $var1^F$) are shown in lanes *d–g*, respectively. These diploid segregants were sporulated and haploid products representing each of the four *var1* forms were obtained with the appropriate nuclear markers so that all possible pairwise crosses could be carried out. In addition, the mitochondrial marker configuration in each case was such that the *ery* and *oli1* alleles could be scored. Lanes *h–l* represent the mitochondrial translation products of a sample of random diploid progeny issued from the following crosses: lane *h*, $var1^F \times var1^S$; lane *i*, $var1^S \times var1^{IF}$; lane *j*, $var1^S \times var1^{IS}$; lane *k*, $var1^{IS} \times var1^F$; lane *l*, $var1^{IF} \times var1^F$; lane *m*, $var1^{IF} \times var1^{IS}$.

(Mw 44,000) and the other cap^s *var1* (Mw 40,000), were mixed in different ratios and each mixed cell population labelled with $^{35}SO_4^{2-}$ in the presence of cycloheximide and products of mitochondrial protein synthesis displayed on SDS–polyacrylamide gels. Densitometer tracings of autoradiograms of the gel were analysed to determine the ratios of the two different molecular weight forms of var1 polypeptide; these were then compared to the input ratios of cell types determined by the proportion of cap^r to cap^s cells. As shown in Fig. 2, there is an excellent linear relationship between the proportion of the two cell types as determined by the *cap* marker and the proportion of var1 polypeptides, thus validating the assay of different forms of the protein in mixed cell populations.

Table 1 The generation of new forms of var1 polypeptide does not depend on outside marker (*ery*, *oli1*) exchange

Genotype	% of total diploids	% of output $var1^S + var1^F$	 $var1^{IF}$
Parental (ery^r oli^s + ery^s oli^r1	71	68	32
Recombinant (ery^s oli^s + ery^r oli^r1)	29	73	27

Homoplasmic diploid segregants were isolated from the cross 5DSS (ω^+ ery^r oli^s $var1^S$) × 195D (ω^+ ery^s oli^r1 $var1^F$) and scored for the *ery* and *oli1* loci. 200 segregants of each parental (ery^r oli^s, ery^s oli^r1) and recombinant (ery^r oli^r1, ery^s oli^s) genotypes were obtained and the percentage of each *var1* type among the progeny was quantitated using a mixed population assay (Fig. 2).

Non-parental forms of var1 polypeptide appear in the progeny of some crosses

Analysis of the transmission of the var1 gene in crosses between strains with different var1 alleles led to the unexpected finding that in some crosses, progeny with non-parental forms of var1 polypeptide are obtained. The most striking example is seen in the random diploid progeny from the cross between strains 5DSS and 195D (Fig. 3) which carry respectively, the largest (44,000) and the smallest (40,000) molecular weight forms of the protein detected thus far. For ease of discussion, we refer to the 44,000 molecular weight species as the S (slow) form and the 40,000 molecular weight species as the F (fast) form.

In the random diploid progeny obtained from this cross, about 30% of the total var1 polypeptide occurs as a non-parental form, with a mobility intermediate between the two parental types, corresponding to an apparent molecular weight of 41,800 (Fig. 3). We subcloned the mixed progeny of the cross and scored *var1* alleles in 60 homoplasmic diploid clones. In addition to the two parental and the 41,800 molecular weight forms, we found two clones with another non-parental form of var1 polypeptide of molecular weight 42,200. Examples of the two parental and two non-parental types of homoplasmic diploid progeny are shown in Fig. 3. These new forms of var1 polypeptide are designated IF (intermediate, fast) for the 41,800 species and IS (intermediate, slow) for the 42,200 species. These are the only non-parental forms of var1 polypeptide that we have observed among 300 individual diploid progeny analysed from this cross.

The generation of new forms of var1 polypeptide does not depend on outside marker exchange

For most mitochondrial genes, separation of pairs of alleles by roughly 10,000 base pairs or more is sufficient to yield a recombination frequency of 20–25%, the upper limit generally being observed. The formation of IF is striking, as it occurs at about that frequency even though the structural gene is contained within a DNA segment no greater than about 2,000 base pairs[16] (R.D.V. *et al.*, unpublished data). If IF and IS arise by recombination, we could account for the data of Fig. 3 by invoking high-frequency reciprocal recombination, for example, at a hot spot within the gene. Alternatively, high-frequency unequal crossing-over or a gene conversion event in which one or both of the parental forms is converted to the new allele at a high rate, could also account for the data.

Because IF and IS are not obtained in equal proportions (about 30% and 2%, respectively) it is unlikely that they are mainly the products of a reciprocal recombination event; this conclusion is supported by the observation that in mixed cell cultures, there is no difference in the growth rates of cells that correlates with *var1* genotype. Unequal crossing-over is also an unlikely mechanism to account for our results as we have never observed new forms of var1 polypeptide in crosses of the type F×F or S×S.

We have examined whether the generation of non-parental forms of var1 polypeptide depends on outside marker exchange. We repeated the cross between strains 5DSS and 195D and selected homoplasmic diploid segregants for each of the four possible genotypes involving the *ery* and *oli1* drug markers which flank the *var1* locus. These are the parental genotypes *ery*^{r} *oli*^{s} and *ery*^{s} *oli*^{r}*1* and the recombinant genotypes *ery*^{r} oli^r*1* and *ery*^{s} *oli*^{s}. To facilitate the analysis, we pooled the individual diploid segregants according to genotype into groups of 20. Ten such groups (of each genotype) were then analysed for the relative distribution of the S, F and IF forms of var1 polypeptide. IS is not generated at a sufficiently high frequency to allow quantitation in this experiment.

The data in Table 1 show that IF comprises about the same fraction of the total *var1* species in either the parental or recombinant classes of progeny. Hence, we can conclude that the generation of IF is independent of any reciprocal crossing-over between its flanking markers. We have also observed that recombination at the *var1* locus is unaffected by interactions of ω^+ and ω^- in heteropolar crosses (data not shown); however, all the crosses reported here are of the homopolar ($\omega^+ + \omega^+$) type.

Non-parental forms of var1 polypeptide arise by gene conversion

Gene conversion can be detected as the loss of one parental allele with a concomitant increase in recovery of the other parental allele or of a recombinant form. To determine if new forms of var1 polypeptide arise by a process of gene conversion, we compared the transmission of the parental alleles with that of the *ery* and *oli1* drug markers. Because, in homopolar crosses, drug markers are recovered coordinately among the progeny, any gene conversion events involving *var1* could be detected as a deviation from coordinate transmission with *ery* and *oli1*. As shown in Table 2 (cross 1) (see Fig. 3), S was transmitted, within experimental error, coordinately with *ery*^{r} and *oli*^{s}. On the other

Fig. 4 *Hinc*II + *Bam*HI restriction endonuclease pattern of mtDNA. mtDNA was prepared from ρ^+ strains 5DSS, 195D and recombinant diploid progeny derived from their mating (see Fig. 3). The mtDNA of each strain was restricted with *Hinc*II + *Bam*HI and electrophoresed on a 2% agarose slab gel for 890 V h, stained for 10 min with 0.5 mg ml^{-1} ethidium bromide and photographed under UV illumination (256 nm). In these electrophoretic conditions, the small *Bam*HI fragment generated from *Hinc*II-10 is not shown because it runs off the gel. Lanes *a* and *h*, ΦX174-RF DNA (combination of digests with *Hpa*II and *Hae*III); lanes *b-g*, mtDNA digested with *Hinc*II + *Bam*HI; lane *b*, strain 5DSS; lane *c*, diploid *var1*^{S}; lane *d*, diploid *var1*^{IS}; lane *e*, diploid *var1*^{IF}; lane *f*, diploid *var1*^{F}; lane *g*, strain 195D. The arrow indicates the large *Hinc*II-10 + *Bam*HI fragment.

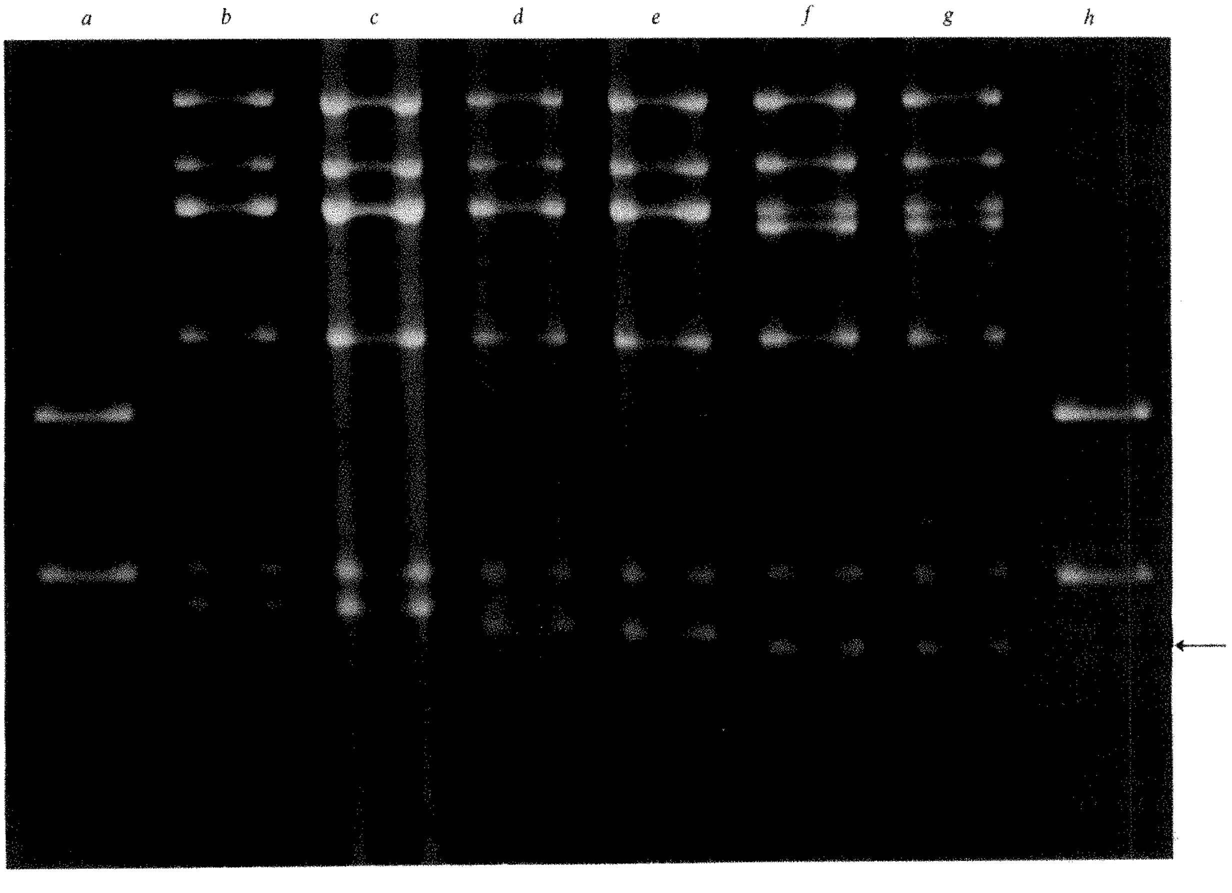

Table 2 Quantitative transmission of *var1* and antibiotic markers in crosses

				% Transmission					
Cross	*ery*^{s} *oli*^{r}*1*	×	*ery*^{r} *oli*^{s}	*ery*^{s}, *oli*^{r}*1*	*ery*^{r}, *oli*^{s}	*var1*^{F}	*var1*^{IF}	*var1*^{IS}	*var1*^{S}
1	F	×	S	53	47	27(53)	30	2	40(47)
2	F	×	IF	60	40	29(60)	71(40)	—	—
3	F	×	IS	62	38	61(62)	—	39(38)	—
4	IF	×	S	52	48	—	51(52)	—	49(48)
5	IS	×	S	43	56	—	—	21(43)	79(56)
6	IF	×	IS	38	62	—	39(38)	39(62)	22

The progeny of crosses described in Fig. 3 were scored for transmission of the *var1*, *ery* and *oli* loci. *Var1* transmission was quantitated according to the protocol described in Fig. 2. In each case, the cross was repeated several times and the values given are means. The expected transmission values for *var1* based on coordinate transmission with the drug markers, are given in parentheses The dash indicates that no *var1* of that type was observed in random diploid progeny.

hand, compared to the transmission of *ery*^{s} and *oli*^{r}*1*, only about one-half of the input F alleles were recovered in the output of the cross. Importantly, this loss could largely be accounted for by the amount of the IF species which occurred in the diploid progeny, suggesting that this new form of *var1* arises chiefly by a gene conversion of the F allele. As the frequency of production of the IS species is less than 2%, it is difficult from this type of experiment to determine whether it also arises by a gene conversion event. Although the transmission of S is within two standard deviations of that of *ery*^{r} and *oli*^{s}, every estimate of S transmission was numerically smaller than that of the drug makers; thus, it is possible that there is some net loss of S so that reciprocal recombination could play a minor part in the generation of non-parental forms of *var1*.

To examine this possibility and to determine whether new forms arise only when parental genomes pair, diploids representing each of the four forms of var1 polypeptide were sporulated and the appropriate mating types and nuclear markers obtained in the haploid products to permit all possible pairwise matings involving the four forms of the var1 polypeptide. In Table 2, cross 2, we see that when F and IF are mated, both *var1* alleles are transmitted non-coordinately with the drug markers; we observed low transmission of F and high transmission of IF. This result helps to explain the frequency of IF production in cross 1 (Table 2), as IF genomes formed in the first round of recombination between parental genomes can pair with F genomes to yield more IF in subsequent pairings.

The results of all the other pairwise crosses are shown in Fig. 3 and Table 2, crosses 3–6. The results were clearcut in that no gene conversion was observed in the crosses F×IS or IF×S, whereas conversion was observed in the crosses IS×S and IF×IS. In the latter two cases, IS was converted to S. Note that in the case of cross 6, the conversion of IS to S represents, insofar as this particular cross is concerned, the formation of a non-parental form of *var1*. This result shows clearly that the gene segments which distinguish IF from IS are non-overlapping and leads to the conclusion that F and S differ from each other in at least two distinct regions.

As indicated above, IS could arise in cross 1 by gene conversion or by crossing-over. If the latter were true, then the frequency of crossing-over within the *var1* gene would be of the order of 2%, that is, the frequency of occurrence of IS in cross 1. This would mean that in cross 6, F should then be present in roughly 2% of the total progeny. To obtain greater sensitivity, we analysed an additional 200 random diploid progeny of cross 6 in 20 pooled groups of 10. Again, no F was detected, suggesting that if there are any F progeny in cross 6, they are produced less frequently than IS in cross 1. These data strongly suggest that in the original F×S cross, IS arises by a gene conversion event, but one less frequent than that responsible for the production of IF.

Non-parental forms of var1 arise by physical exchange of DNA sequences

We next extended our analysis to the segment of mtDNA containing the *var1* gene. As noted above, we have succeeded in locating the *var1* gene to a specific *Hin*cII + *Bam*HI restriction endonuclease fragment, about 2×10^{3} base pairs long located between *ery* and *oli1* (Fig. 1), the absolute size of which is strain dependent and varies according to the size of the var1 polypeptide which it encodes[16] (R.D.V. *et al.*, unpublished data).

We isolated mtDNA from homoplasmic diploid progeny of cross 1 (Table 2) representative of each of the four forms of var1 polypeptide and digested it with *Hin*cII and *Hin*cII + *Bam*HI. As indicated in Fig. 1, *Hin*cII fragment 10 (*Hin*cII-10) contains one *Bam*HI site. The structural gene for var1 polypeptide is contained wholly within the larger *Hin*cII-10 + *Bam*HI fragment[16] (R.D.V. *et al.*, unpublished data). Figure 4 shows representative restriction digests of mtDNA from each of the four diploid clones with the parental and non-parental forms of var1 polypeptide. The gel was electrophoresed long enough to accentuate the difference between the large *Hin*cII-10 + *Bam*HI fragments. The small *Hin*cII-10 + *Bam*HI fragment was run off the gel; its molecular weight has been determined on separate gels run for a shorter time. The data show quite clearly that the large *Bam*HI fragment of *Hin*cII-10 varies in size according to the size of the var1 polypeptide for each strain with a different molecular weight form of the protein.

These results are summarised quantitatively in Table 3, which contains our estimates of the molecular weights in the different strains of the *Hin*cII-10 and *Hin*cII-10 + *Bam*HI fragments. Within experimental error, the sizes of the large *Bam*HI fragment of *Hin*cII-10 in the diploids with the S and F forms of var1 polypeptide are the same as those in the parental haploid strains 5DSS and 195D, respectively. The fragment is larger in IS than in IF strains and both are intermediate in size between the S and F forms. Without exception, therefore, a change in the size of var1 polypeptide is accompanied by a change in the size of the mtDNA fragment containing its structural gene.

We have also analysed mtDNA from progeny of the IS×IF cross where the parental S form is generated (Fig. 3). In this case,

Table 3 Apparent molecular weights of mtDNA fragments from ρ^{+} strains with different *var1* alleles

Strain	*Hin*cII band 10	*Hin*cII band 10 + *Bam*HI	
		Large fragment	Small fragment
5DSS	1.872 ± 0.006	1.438 ± 0.005	0.428 ± 0.001
S	1.842 ± 0.008	1.435 ± 0.007	0.427 ± 0.001
IS	1.842 ± 0.007	1.409 ± 0.009	0.427 ± 0.003
IF	1.813 ± 0.004	1.395 ± 0.007	0.406 ± 0.002
F	1.788 ± 0.009	1.376 ± 0.008	0.405 ± 0.002
195D	1.790 ± 0.008	1.377 ± 0.008	0.406 ± 0.002

mtDNA from ρ^{+} strains 5DSS and 195D and recombinant diploid progeny containing parental (S, F) or non-parental (IS, IF) forms of *var1* polypeptide were analysed as in Fig. 3. Apparent molecular weights of *Hin*cII band 10, or the fragments generated from it by digestion with *Bam*HI, were calculated by comparison with the mobilities of ΦX174-RF DNA fragments (combination of digests with *Hae*II, *Hae*III and *Hpa*II) run in adjacent lanes on slab gels containing 2% agarose. *Hin*cII digests were run for 850–900 V h; *Hin*cII + *Bam*HI digests for 630 V h to measure the small fragment of *Hin*cII band 10, or 850–900 V h to measure the large fragment. The small fragment runs off the gel when maximal separation of the large fragments is obtained. Each value represents the mean of 4–7 independent gel measurements

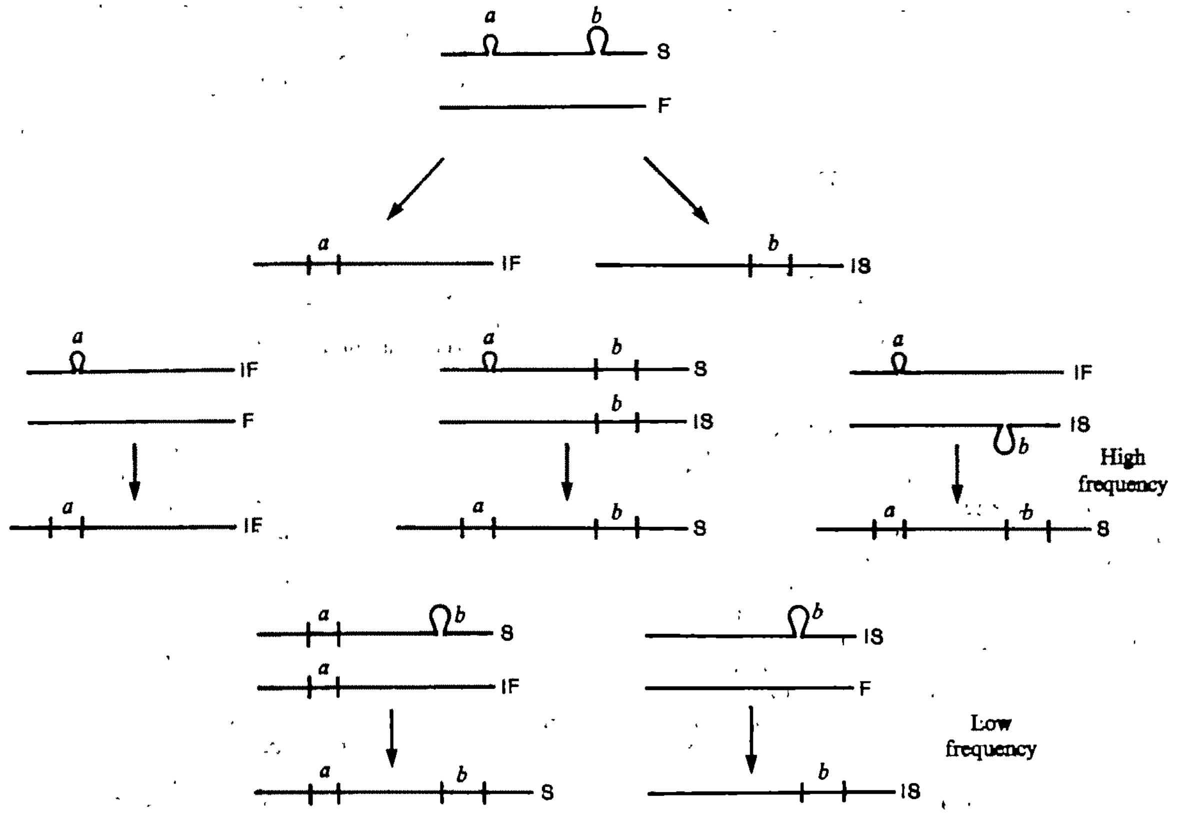

Fig. 5 A model for asymmetric gene conversion at the *var1* locus. Heteroduplex pairing of DNA segments from different *var1* alleles is shown; the positions of the looped out portions are arbitrary.

the size of the *Hin*cII-10+*Bam*HI large fragment in the S diploids issued from this cross is indistinguishable from the parental (5DSS) S form (data not shown). This result, and the fact that the sum of the molecular weight differences between IF, IS and F equals the molecular weight difference between S and F, provide additional support to our conclusion that the regions of difference between IF and IS are non-overlapping.

A model for gene conversion at the *var1* locus

Figure 5 presents a simple scheme fully consistent with the genetic and biochemical data presented here, showing how non-parental forms of the *var1* gene and its product may arise by a process of asymmetric intragenic gene conversion. The model shows two DNA segments or insertions within the S gene which are absent from the F gene. From molecular weight determinations of the parental and non-parental DNA fragments which contain the *var1* gene, we can estimate the lengths of the inserted segments, designated *a* and *b*, to be 36 and 57 base pairs, respectively. Subsequent to heteroduplex formation, these segments are copied independently into the adjacent shorter heteroallele. In the heteroduplex formed between the S and F parental alleles, gene conversion is initiated, perhaps by a single strand break in the (short) strand opposite insertion *a* or *b*, and completed by gap formation and gap-filling repair replication.

Although the two insertions are rather similar in size, our data clearly show that the efficiency of execution (probably of the initial step of the gene conversion event) is much greater for segment *a* than for segment *b*. Not only is IF the most abundant non-parental form of var1 polypeptide issued in the original F×S cross, but also in subsequent backcrosses, in all pairwise matings which could yield a heteroduplex between a strand carrying insertion *a* and one lacking it, the latter is frequently converted to a form containing the *a* insertion. As shown in Figure 5, this may be seen either as the generation of a non-parental form by conversion of one of the parental alleles (such as in the cross IF×IS) or as an unequal transmission of the parental forms (as in the crosses between IF×F and IS×S). On the other hand, backcrosses giving rise to heteroduplex pairs where both strands either contain or lack insertion *a*, although heteroallelic or homoallelic for insertion *b*, show little change in the output of the parental forms. This result is entirely consistent with the observation that the formation of IS, which formally represents the insertion of segment *b* into F in the S×F cross, occurs at a frequency of less than 2%.

The model presented here bears a similarity to the events suggested to occur at the ω locus in heteropolar ($\omega^+ \times \omega^-$) crosses[4,9]. Gene conversion at ω was proposed to be a fundamental feature of mitochondrial transmission genetics[7,8]. It was further suggested that gene conversion might be a general feature of recombination in yeast mtDNA. As is the case at the ω locus, this could be asymmetric, giving rise to a gradient in transmission of markers and grossly unequal numbers of reciprocal recombinant genotypes, or symmetric, in which case coordinate transmission of markers and equal numbers of reciprocal recombinant types would be obtained. Since that time, the analysis of recombination in other regions of the genome failed to reveal additional examples of efficient polar recombination[19,20] so that the events at ω were then viewed as an interesting but special case. We are prompted by our present results to reappraise the relative role of gene conversion as an important feature of the inheritance of mitochondrial genes.

The high frequency of the recombination (gene conversion) events at *var1* described here and those at other regions of the mitochondrial genome, may be explained as a consequence of multiple rounds of recombination in each cell generation[4]. Although that is almost certainly an important property of yeast mitochondrial genetics, it is possible that site-specific recombination may also be involved. In particular, insertions or base sequences at the ends of insertions, may play an important part in mitochondrial recombination. It will be of great interest to learn whether any of these insertions have structural features or base sequences in common and to extend the analysis of the mode of inheritance of other insertions present in mtDNA from various yeast strains to those both larger and smaller than ω^+.

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Mycoplasma capping on lymphocytes

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When mycoplasmas infect lymphocytes they behave as multivalent ligands and cap on the lymphoid cell surface in the absence of added specific antibody. There is an apparent high correlation between mycoplasma capping and blast transformation of the infected lymphocytes. Mycoplasma caps are shed from the surface of cells as an aggregate containing host membrane vesicles. This novel interaction may suggest a physiological role for the phenomenon of capping and may play a part in mycoplasma pathogenesis.

MYCOPLASMAS are extracellular parasites often found in close association with the host cell plasma membrane[1,2]. Their predilection for cell surfaces, especially moist mucosa, is responsible in part for their involvement in pathological states such as chronic respiratory disease, polyarthritis and genital tract infections in many species of animals. Little is known about the specific nature of mycoplasma–host cell interactions either *in vivo*, where the parasites have a fairly strict host species specificity, or *in vitro*. Although specific cell receptors have been described for a few mycoplasma species[3–5], their chemical identities for the most part remain obscure.

We have investigated the nature of these interactions *in vitro* and their possible relevance to the pathogenic consequences of mycoplasma infection[6,7]. We describe here a novel interaction between mycoplasmas and lymphocytes *in vitro*, in which the mycoplasmas behave as multivalent ligands and cap on the surface of infected lymphocytes in the absence of specific antibody.

Mycoplasma infection of an established lymphoblastoid line

Using a strain of *Mycoplasma hyorhinis* adapted to rapid cell colonisation, we infected a mouse lymphoma line, S49 (ref. 8), with mycoplasmas at multiplicities of infection of 1–10 viable organisms per cell. Infected cells were grown in suspension in RPMI medium containing 10% calf serum at 37 °C and then processed for electron microscopy and immunofluorescence 1–2 d after infection.

Viable control and infected cells were stained with fluorescein-conjugated anti-*M. hyorhinis* antiserum diluted in isotonic buffered saline. To compare the distribution of mycoplasmas on the surface of lymphocytes as a function of the lateral mobility of their receptors in the fluid membranes[9–11], antibody staining was carried out at room temperature and 0 °C, and also on lymphocytes poisoned with 5×10^{-3} M sodium azide just before staining. To establish the rate of appearance of caps, S49 cells were stained with fluorescein-conjugated anti-mycoplasma antibody at intervals during a 24-h period after infection with *M. hyorhinis*.

Cells were also fixed in glutaraldehyde and then post-fixed in osmium tetroxide. After dehydration and embedding in resin, thin sections were cut, stained and examined in a Philips 300 electron microscope.

Mycoplasma infection of mouse spleen cells

Spleens were removed from 6–8-week-old BALB/c and *nu*/*nu* (nude) athymic mice, and the spleen cells suspended in RPMI medium containing 10% calf serum. After treatment with ammonium chloride to lyse erythrocytes, the spleen cells were seeded at approximately 5×10^6 per ml in RPMI medium containing 10% calf serum and 5×10^{-5} M 2-mercaptoethanol.

Table 1 Rate of mycoplasma capping on S49 cells

Time after mycoplasma infection (h)	% Cells infected		% Cells capped		% Cells patched	
	PBS	5×10^{-3} M NaN_3	PBS	5×10^{-3} M NaN_3	PBS	5×10^{-3} M NaN_3
1	0.65	0.58	0.0	0.01	0.65	0.57
4	6 40	6.62	2.80	2.65	3 60	3.97
8	17.66	21 20	7.85	8.40	9.81	12 80
16	22.60	22.57	19.20	18.50	3.40	4 07
24	43.35	40 46	38.23	35.80	5.12	4 66

S49 lymphoblastoid cells (3×10^6 cells ml^{-1}) were infected with *M. hyorhinis* at 5×10^7 colony-forming units per ml. At the indicated time intervals cells were washed with phosphate-buffered saline (PBS), stained with fluoresceinated anti-*M. hyorhinis* antiserum diluted in PBS, and mounted in 1:1 PBS:glycerol. The preparations were then scored for cells exhibiting specific fluorescence. At least 1,000 cells were scored for each determination. Cells treated with 5×10^{-3} M sodium azide (NaN_3) were exposed to this inhibitor during the entire process of washing, staining and mounting. In addition, these azide-treated cells were processed at 0 °C. Untreated cells were processed identically except that NaN_3 was omitted from the reagents and the manipulations were carried out at room temperature.

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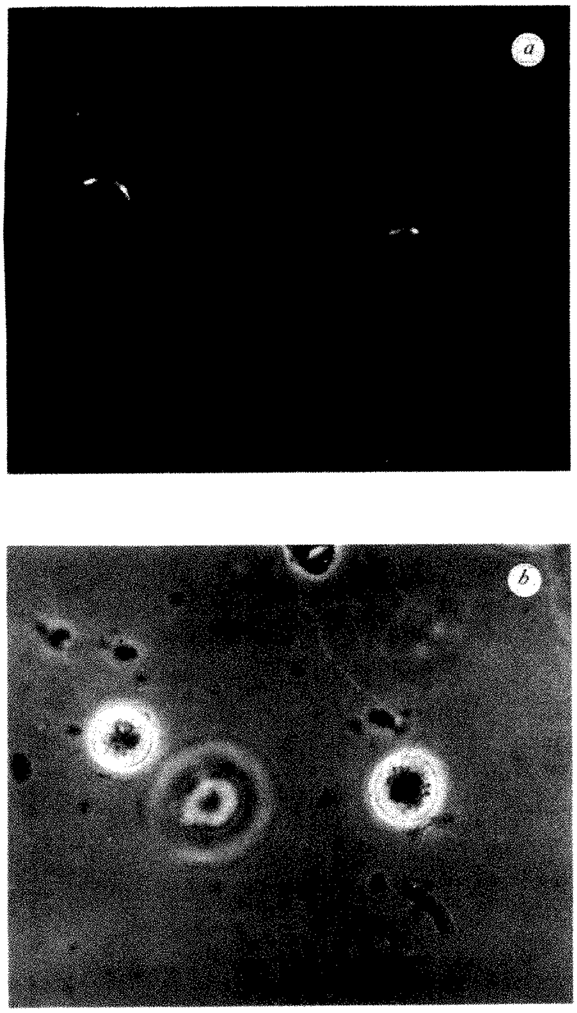

Fig. 1 S49 lymphoblastoid cells infected with *M. hyorhinis*. *a*, Capped mycoplasmas revealed with fluoresceinated anti-mycoplasma antiserum 24 h after infection. Magnification, ×1,200. *b*, Phase microscopy of the same field. Magnification, ×1,200.

Cells were then infected with $1-5\times10^7$ colony-forming units of *M. hyorhinis* and incubated at 37 °C. Cultures were taken daily for 3 d and blast formation was scored using phase optics according to the number of blast cells in a given lymphocyte population.

The number of dead cells was estimated by staining the unfixed populations with 0.5 μg ml^{-1} 4′-6-diamidine-2-phenylindole (DAPI), which enters dead cells and binds specifically to DNA. Thus, the nuclei of dead cells fluoresced when examined microscopically using the Leitz Ploem system whereas those of live cells did not. The degree of blast transformation was confirmed by fixing cells with 0.5% glutaraldehyde on ice for 10 min followed by staining with Jenner–Giemsa stain. Cap formation was monitored by immunofluorescent staining as described above. For comparison, parallel cultures of splenic lymphocytes were treated with 10 μg ml^{-1} of bacterial lipopolysaccharide (LPS), a potent mitogen for B lymphocytes[12].

We did not attempt to measure lymphocyte stimulation by the conventional thymidine incorporation assay[16] because our preliminary experiments (unpublished) gave highly variable results due to the nucleoside phosphorylase activity of mycoplasmas which catalyses the rapid conversion of thymidine to thymine[17,18].

Mycoplasma capping on S49 cells

The rate of formation of mycoplasma caps on the S49 lymphoblastoid cells is shown in Table 1. Initially, fluorescence was seen in very few cells and was predominantly distributed in an irregular patched pattern over the cell surface. Several hours after infection, the frequency of caps increased (Fig. 1), reaching a peak approximately 24 h after infection. There was no significant difference in the frequency of mycoplasma caps in the presence or absence of 5×10^{-3} M sodium azide, which is an inhibitor of respiration and consequently of energy-dependent capping[11]. This indicates that the mycoplasmas were behaving as multivalent ligands independent of specific anti-mycoplasma antibody. The kinetics of appearance of the caps after being distributed over the entire cell surface, as evidenced by early ring and patch fluorescence followed by capping, indicated a process of interaction and movement similar to that seen with ligands such as concanavalin A (refs 13, 14), albeit slower in rate.

Capping in the absence of specific antibody was clearly established in our ultrastructural studies. As Fig. 2 shows, mycoplasmas were commonly situated at one pole of a cell when examined by transmission electron microscopy. Scanning electron microscopy (Fig. 3) showed the polar accumulation of capped mycoplasmas particularly distinctly. Accumulation was often sufficient to deform the lymphocytes physically, and was also seen in the phase and immunofluorescent preparations shown in Fig. 1. Uropods formed at the sites of mycoplasma accumulation (Fig. 4), and the mycoplasmas clustered around the uropod were often associated with small membranous vesicles, presumably derived from the host cell surface membrane. Towards the latter stages of the 24-h infection period the mycoplasmas were shed from the surface of the lymphocytes as large aggregates containing the membranous vesicles of

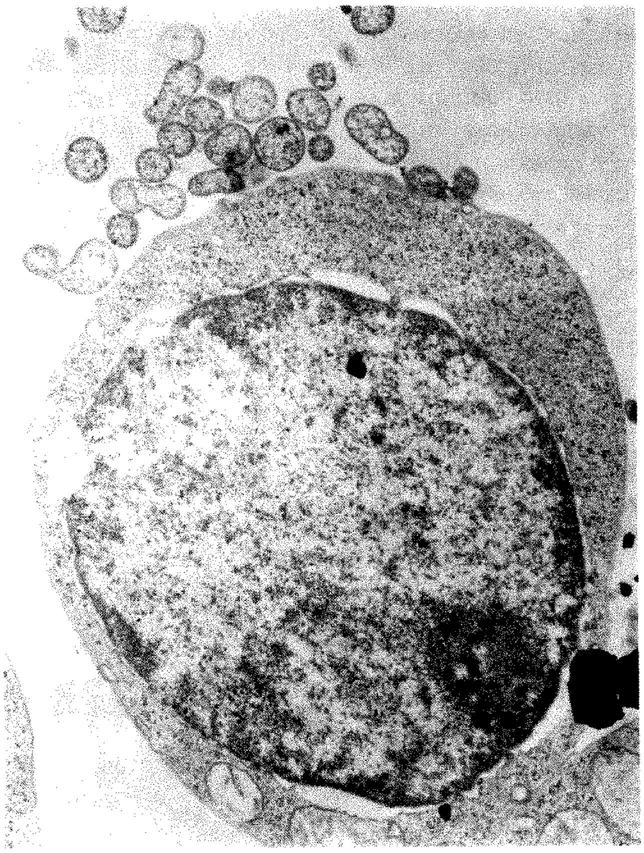

Fig. 2 S49 lymphoblastoid cell infected with *M. hyorhinis*. A cap of mycoplasmas is located at one pole of the cell. Cells were chilled on ice, washed once in 0.2 M Na cacodylate buffer, *p*H 7.2, fixed in the same buffer continuing 3% glutaraldehyde for 1 h, pelleted and fixed directly in 1% osmium tetroxide in Veronal buffer (*p*H 7.2; 0.5 mM $CaCl_2$). The pellet was then dehydrated in ethanol. Fixed cells in 100% ethanol were then warmed to room temperature, infiltrated with resin and further processed as described in the text. Magnification, ×18,000.

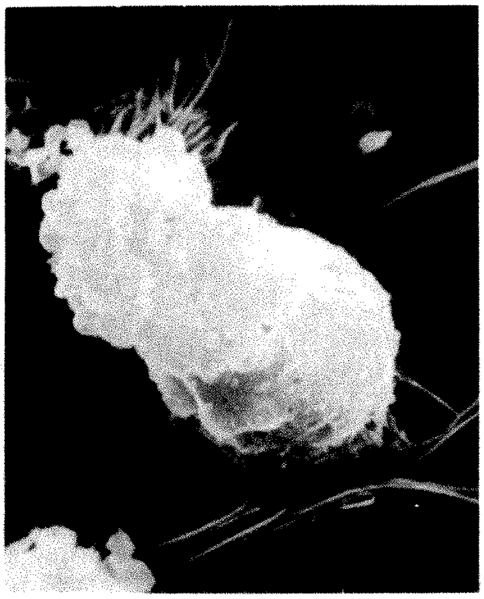

Fig. 3 Scanning electron micrography of an S49 lymphoid cell with a prominent cap of mycoplasmas. Fixation was accomplished by diluting with 0.2 M potassium phosphate buffer containing 2.5% glutaraldehyde, pH 7.5, 1 : 1 with medium, at room temperature. Cells maintained their original shapes at this dilution. The cells were washed with 0.1 M potassium phosphate buffer, pH 7.4, and allowed to attach to the surface of coverslips on which a thin layer of 1% poly-L-lysine (MW 70,000) had been dried[34]. Dehydration in ethanol was followed by transfer to acetone and critical point drying. Magnification, ×7,900.

presumed host origin. We saw no evidence of endocytosis, which is often seen when surface antigens are capped by antibodies[14,15]. In immunofluorescent preparations, shed caps appeared as distinct, freely floating, highly fluorescent aggregates.

Blast transformation of mouse spleen cells

M. hyorhinis behaved as a potent mitogen for both BALB/c and *nu/nu* splenic lymphocytes. Table 2 shows that the mycoplasmas were more effective than LPS in stimulating blast transformation. Although blast cells were not scored as such until obvious morphological changes had taken place, we noted subjectively that 'preblasting' (that is, the appearance of medium lymphocytes) occurred earlier and involved more cells in the mycoplasma-infected cultures than in the LPS-treated cultures, particularly those derived from *nu/nu* spleens.

Examination of infected cells by immunofluorescence revealed a series of events (Table 3) similar to that observed in S49 cells. Within 24 h of infection, approximately 40% of cells showed evidence of infection as measured by specific fluorescence. Most of these cells had distinct fluorescing caps and the remainder exhibited patchy fluorescence. Relatively few blast cells were visible but virtually all were capped. A significant number of blast cells had small pinpoints of specific fluorescence at a single pole of each respective cell, especially in the *nu/nu* cultures. By the second day of infection the number of blast cells with polar pinpoints of infection had increased considerably. In *nu/nu* spleen cultures in particular, many blast cells were seen which had no membrane-associated fluorescence; by day 3 most *nu/nu* blast cells failed to exhibit specific fluorescence.

We consider these series of observations to indicate that the process of infection, patching and capping is closely related to the induction of blast transformation in these cells. Furthermore, the caps seemed to be shed from the surface of the blasts, and reinfection of the stripped cells did not recur during the time of observation. Very few of the unstimulated or dead cells on days 2 or 3 were infected, as measured by specific immunofluorescence.

Significance of mycoplasma capping

The phenomenon of capping, first described by Taylor *et al.*[11], convincingly demonstrated that cell surface receptors which are normally distributed randomly in the plane of the membrane may be redistributed into patches and caps after crosslinking by multivalent ligands. This original study and subsequent reports[14,15,19] demonstrated capping by crosslinking surface antigens with antibodies directed against the surface antigens or by using soluble ligands such as concanavalin A (refs 13, 20). We believe that the studies described here are the first report of an infectious organism behaving in a fashion similar to soluble multivalent ligands and of capping on the surface of lymphocytes in the absence of specific antibody. Certain viruses are distributed in patches on the surface of lymphocytes in the absence of antibody, but the addition of specific antibody is required to induce capping[21].

The association between capping and blast transformation is also particularly striking. We do not yet know if mycoplasma capping is causally related to lymphocyte stimulation, but if this proves to be the case it may portend a physiological significance for capping during microbial infection. Several mycoplasma species have been shown to induce blast transformation of splenic lymphocytes *in vitro*[22-25] and result in polyclonal B-cell activation[26]. It will be interesting to see if there is a general

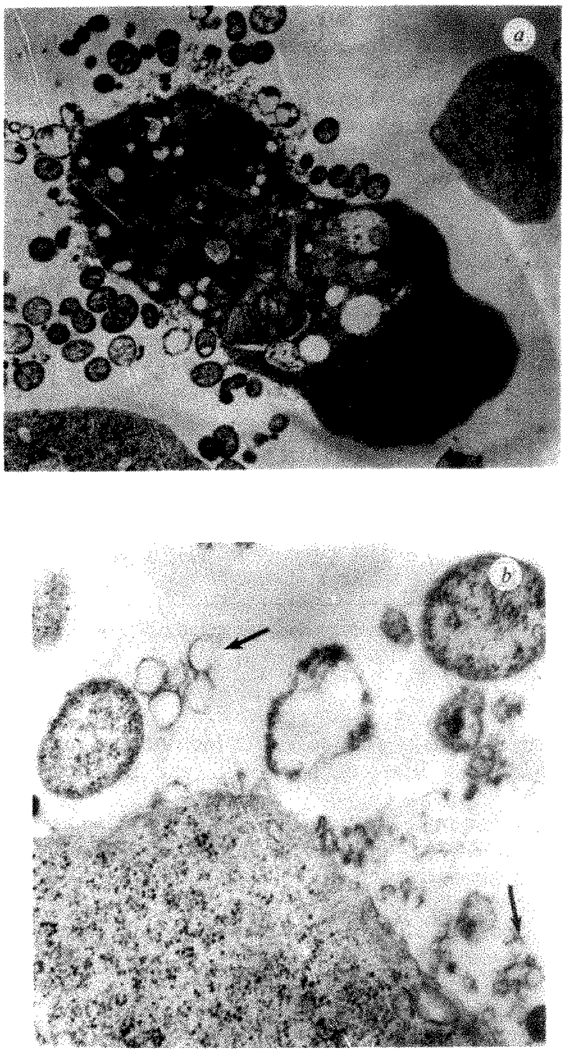

Fig. 4 Mycoplasma caps on S49 lymphoblastoid cells. *a*, Thin section of lymphocyte containing a broad cap of mycoplasmas associated with a uropod. Magnification, ×8,900. *b*, Higher magnification of a uropod showing membrane vesicles (arrowed) released from the cell surface. Magnification, ×40,000.

Table 2 Comparative mitogenic effects of bacterial lipopolysaccharide and mycoplasmas on mouse lymphocytes

Time post-treatment or infection (d)	Treatment of cells	BALB/c splenic lymphocytes: Total cells counted	% Blast cells	% Dead cells	*nu/nu* splenic lymphocytes: Total cells counted	% Blast cells	% Dead cells
	Control	483	0.6	24.6	503	1.3	24.4
1	LPS	509	1.9	25.5	503	8.1	22.0
	M. hyorhinis	459	4.3	26.3	444	9.2	26.8
	Control	446	0.4	31.1	380	1.5	36.2
2	LPS	402	15.1	23.8	350	22.7	22.4
	M. hyorhinis	429	37.0	34.9	362	38.3	36.7
	Control	350	0.6	51.8	351	7.1	36.3
3	LPS	367	27.5	23.6	355	29.5	23.3
	M. hyorhinis	356	41.2	49.2	384	52.6	41.6

The blastogenic transformation of the splenic lymphocytes was scored using phase optics and confirmed following fixation and staining with Jenner–Giemsa. Dead cells were determined by DAPI staining as described in the text. Blast cell percentages were calculated as per cent of total cells.

correlation between mycoplasma capping and lymphocyte activation with these other species. The strong correlation between mycoplasma caps and blast transformation suggests that this may well be the case.

Our preliminary results presented here also indicate that mycoplasmas seem to be a more potent mitogen than LPS. The isolation of the active mycoplasma components will clarify this possibility. The fact that both BALB/c and athymic nude splenic lymphocytes were stimulated suggests that the predominant cells affected are B lymphocytes. We have confirmed this using anti-immunoglobulin sera but have also found that a proportion of the stimulated cells do not bear immunoglobulin (Ig) on their surface (unpublished observations). We do not yet know if these represent a population other than B cells or whether surface Ig has been co-capped with the mycoplasmas. Our preliminary results with partially purified T cells indicate that they too are stimulated to a lesser degree (E.J.S. and B. Pires, in preparation).

Table 3 Relationship between mycoplasma capping and blast transformation

Time after mycoplasma infection (d)	BALB/c splenic lymphocytes: % Cells infected: Total	Capped	Patched	Blast cells	% Blast cells infected: Total	Capped	Patched	*nu/nu* splenic lymphocytes: % Cells infected: Total	Capped	Patched	Blast cells	% Blast cells infected: Total	Capped	Patched
1	41.4	33.6 (24.8)	7.8	8.8	89.4	52.6 (15.7)	36.8	32.4	24.8 (20.4)	7.6	14.7	83.7	61.1 (44.4)	22.6
2	60.0	30.2 (18.6)	29.8	49.2	100	62.5 (33.4)	37.5	25.6	22.0 (20.7)	3.6	47.3	25.2	24.0 (24.0)	1.2
3	29.8	24.1 (19.3)	5.7	51.1	46.8	44.5 (30.2)	2.3	15.5	13.7 (12.6)	1.8	58.0	14.0	13.2 (12.6)	0.8

The distribution of mycoplasmas on infected cells was determined by specific immunofluorescence. Percentages in parentheses refer to pinpoint polar capping as discussed in the text.

Certain features of the mycoplasma–lymphocyte interactions described above may be important for our understanding of the pathogenicity of these microorganisms. Mycoplasma infections are usually self limiting but are occasionally followed by complications which are autoimmune in nature[2]. For example, *M. pneumoniae* causes primary atypical pneumonia in humans, a condition which is usually limited to the respiratory tract. However, documented sequelae to this infection include anaemia and neurological complications[2,27,28]. Also, antibodies directed against host tissue, including the I antigen of erythrocytes, lung and brain, have been found in the sera of patients infected with *M. pneumoniae*[28,30]. If mycoplasmas are capable of inducing B-cell activation *in vivo*, as they are *in vitro*, it is possible that 'forbidden' clones may be activated to produce autoantibodies.

An alternative way in which mycoplasma–host cell interactions may lead to autoimmune reactivity is by antigenic modulation. It is well established that during infection certain viruses alter the composition of the host cell's plasma membrane[31,32]. The changes which ensue include particularly the depletion or loss of histocompatibility antigens from the surface[32]. Antigenic modulation of cells following viral infection has been linked to the immunopathology caused by these infections. We have preliminary evidence that infection of mouse lymphocytes with mycoplasmas leads to the depletion of host cell surface antigens, including H-2 (unpublished observations). Similar losses of host antigens following mycoplasma infection of lymphocytes have been noted by other investigators[32]. It is possible that these surface antigens may serve as mycoplasma receptors and are stripped from the cell during the capping process and subsequent release of the cap from the surface of the lymphocyte.

In conclusion, we have shown that a novel feature of the infection of mouse lymphocytes with mycoplasmas is the capping of these organisms at one pole of the cell. Mycoplasma capping is correlated with a high efficiency of blast transformation; thus, mycoplasmas behave as potent mitogens. The caps are shed as aggregates from the surface of the lymphocytes and contain membranous vesicles which are presumably of host origin and may contain mycoplasma receptors. If this interaction has a parallel *in vivo* it may explain some of the autoimmune sequelae to mycoplasma infection.

We feel it is also appropriate to include a final note of warning that investigators using lymphocytes as model systems to investigate mitogenicity, lymphocyte triggering, surface antigens and viral pathology should, together with all other investigators using cultured cells, beware of mycoplasma contamination[1,7].

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letters

HEAO Observations of X-ray bursts from MXB1730−335

BURST activity was observed[1] on 18 March 1978, from MXB1730−335, the rapid burster, from which no activity had been observed a few days earlier[2]. HEAO 1 was pointed (+y axis) at the rapid burster from ~18.00–21.00 UT on 31 March 1978. The source was actively bursting in the 'Mode I' pattern[3], in which very large type II bursts[4] are first followed by a long (several minutes) quiescent period and then by a train of closely spaced, small bursts. Because type I and type II bursts probably have different underlying physical mechanisms[4], it is important to study both types over a large energy range. We report here on a search for hard X-ray emission from the rapid burster and on evidence for spectral evolution in the type II bursts.

Fifty-eight minutes of quick-look data were examined from the A2 experiment and from the A4 experiment on HEAO 1. Two very large bursts were observed in both the A2 and A4 data (Fig. 1). Each lasted ~25 s and had a total energy flux (2–60 keV) of $3\text{–}4\times10^{-7}$ erg cm^{-2}. Five moderately sized bursts (~10 s duration, ~10^{7} erg cm^{-2}) and 167 small bursts (~5 s duration, $2\text{–}5\times10^{-8}$ erg cm^{-2}) were also observed in the A2 data. The two large bursts which occurred at 19 h 12 min 00 s UT and 19 h 48 min 54 s UT, are shown in Fig. 1, as observed in four energy channels from 13–160 keV by the A4 low energy detectors[5] (sum of count rates in two independent detectors each with an area of ~100 cm^2) and in a single, broad energy band, by the A2 detectors. The A2 data shown here are the sum of various energy channels in three separate detectors[6], spanning the energy range 2–60 keV. The A4 data were binned on-board the spacecraft into 0.64 s time bins and ~5 keV energy bins. Background counting rates were determined from ~200 s of data immediately following the 19 h 12 min 00 s burst and from ~35 s of data following a moderate-size burst ~13 min later (not shown in Fig. 1). They include the diffuse X-ray and charged particle background and any steady emission that might come from the rapid burster or from 4U1728−33 which are separated by only ~0.5°.

Because of the steep spectrum of the type II bursts from the rapid burster[3], more than 95% of the total A2 counts is due to photons with energies <13 keV. These data can therefore be considered effectively as an energy channel (2–~13 keV) preceding the first A4 channel (13–25 keV). The large type II bursts from MXB1730−335 in the A2 data seem to be similar to those observed in 1976 (refs 7–9), showing a broad maximum and a double-peaked structure during the burst decay.

Each burst was analysed in two nearly equal time segments. The ratio of counts in the last to the first part of the 19 h 12 min 00 s burst was 0.353±0.004 in the A2 data and <0.15 (3 σ confidence level) in the 13–25 keV channel of the A4 data. No significant detection of X rays was made at all in the A4 data after the first 13 s of the burst, while in the A2 data, at lower energies, the burst lasted ~25 s. The ratio of counts in the

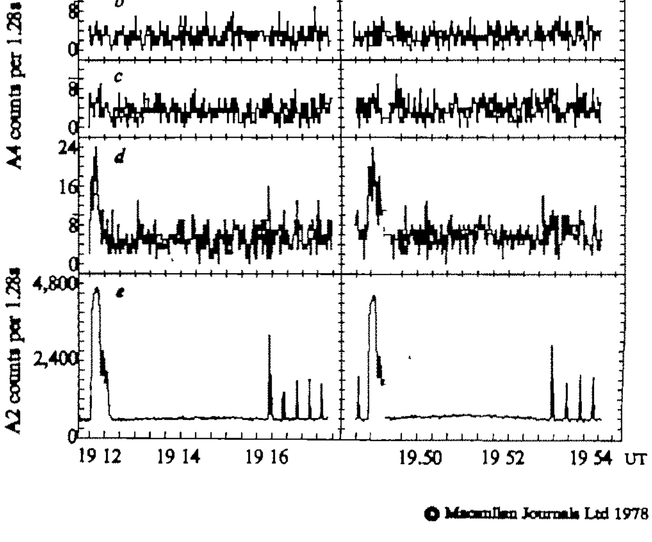

Fig. 1 HEAO 1 data for the rapid burster (MXB1730−335) on 31 March 1978. The enhancement after the large bursts is probably 'steady' X-ray emission as previously observed from the rapid burster[4,19]. *a*, 85–160 keV; *b*, 40–85 keV; *c*, 25–40 keV; *d*, 13–25 keV; *e*, 2–60 keV (95% of counts <13 keV).

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last to the first half of the 19 h 48 min 54 s burst was 0.467 ± 0.006 in the A2 data and 0.30 ± 0.08 in the A4 13–25 keV channel. In both bursts, the X rays with energies above 13keV decayed faster than the lower energy X rays; that is, the spectra softened during the burst decay. The spectral evolution of the bursts can perhaps also be seen from the A2 data alone. In both xenon detectors in which the bursts were observed, the ratios (comparison of second and first half of the bursts) for counts above 9 keV were less than the ratios for counts below 7 keV, with a significance slightly greater than 3 s.d.

We conclude that the type II bursts from the rapid burster do show a spectral softening during burst decay. However, this spectral softening is not nearly as pronounced as in type I bursts, either from MXB1730 − 335 (ref. 4) or from other burst sources[10–13]. Marshall *et al.*[3] found that the spectra of type II bursts from MXB1730–335 as measured by SAS 3 do not change significantly during burst decay. However, the SAS 3 data were not sensitive to the spectral evolution reported here. The highest energy channel with a statistically significant signal in the SAS 3 data was 8–19 keV. For the spectrum of these bursts, this energy channel is dominated by X rays below 10 keV, and the spectral softening reported here could not have been detected.

We searched for the existence of a flux in the 86–160 keV energy range. Combining all the data (corrected for collimator transmission), we find a 90% confidence upper limit[14] of $<1.7\times10^{-8}$ erg cm^{-2} per burst in the range 85–160 keV corresponding to $<2.0\times10^{38}$ erg per burst at a source distance of 10 kpc. The average energy of these two bursts as detected by A2 in the 2–20 keV energy range was $(4.4\pm0.5)\times10^{39}$ erg per burst at 10 kpc. This value is similar to that measured previously for large bursts from the rapid burster[7,8]. Thus large type II bursts from the rapid burster contain <5% of their total X-ray energy in the range 85–160 keV.

This lack of a hard X-ray flux has consequences for those accretion instability models of X-ray bursts which require extremely high temperatures; for example, super-massive black holes ($\sim10^5 M_\odot$) surrounded by a $\sim10^9$ K hot cloud[15] or spherical accretion of compact H II regions on less massive black holes ($\sim$10–100 $M_\odot$)[16]. In the latter, the low energy (<20 keV) burst should be accompanied by at least as luminous a burst in hard X rays (≳100–200 keV). Our present observations, of the absence of any apppreciable flux in the range 85–160 keV, do not, of course, rule out the possibility that a large amount of burst energy is present above 160 keV.

Other evidence has accumulated that sources of type I bursts are not massive black holes but objects of solar mass[9,13,17,18]. As the rapid burster itself is also a source of type I bursts[4], these results for the type II bursts (which are almost certainly due to accretion instabilities) then limit the production of hard X rays that are allowed in the accretion on a neutron star or a small black hole.

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NGC326—a radio galaxy with a precessing beam?

THE radio emission from most strong extragalactic sources is concentrated in a band which passes through, and is symmetrical with respect to, the associated galaxy[1]. This structure is sometimes modified to a V or C shape with the galaxy at the apex, as in the wide angle tail sources like 3C465 (ref. 2), and occasionally to the more extreme elongation of the tail sources such as NGC1265 (ref. 3). In a few sources an S-shaped or 180° symmetry is seen. This was first noted in the outer components of Cen A[4] and a better example is 3C272.1 (refs 5, 6). Other sources where the S distortion is very clear have been found with higher resolution observations (for example Cyg A[7] and 3C47[8]). In these, the structure in question subtends a small angle from the centre and they give a strong impression of a beam type of model in which the beam has wobbled or precessed by a few degrees[9]. A new example of a radio source showing a structure with a 180° symmetry, but not of the S form, has been found as part of a systematic study of radio galaxies of lower luminosity selected from the B2 catalogue[10]. The projection of a precessing beam model seems to provide a natural explanation for this strange shape and is described here.

The radio source B20055+26 (4C26.03) was first mapped with the Westerbork Synthesis Radio Telescope in a program at 1.4 GHz in which short observations were obtained of all the B2 radio sources identified with bright galaxies[11]. Because an interesting structure was seen this was followed up by a 2 × 12 h observation at 5 GHz resulting in a map with resolution 6″ × 13″ (RA × dec) and r.m.s. noise of 0.8 mJy (1 mJy = 10^{-29} W m^{-2} Hz^{-1}) beam area. This map is shown in Fig. 1 and the position of the elliptical galaxy NGC326 is indicated. This galaxy is the brightest member of a small group of galaxies, Zwicky 0056.9+2636, and has a redshift of 0.0472 (ref. 10). There is a fainter elliptical galaxy 2′ NE and the other members of the group are more than 5′ away.

The radio map shows two curving tails with a 180° symmetry. There is also emission at the position of the galaxy. This may be a

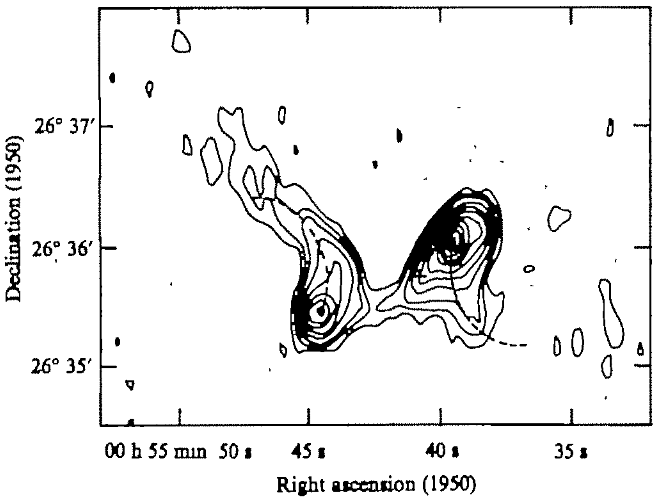

Fig. 1 5.0 GHz map of the brightness distribution of B2 0055+26. Contours are 2, 4, 6, 8, 10, 15, 20, mJy per beam area. The r.m.s. noise level is 0.8 mJy per beam area. 1 mJy per beam area = 0.53 K. The position of the galaxy NGC326 is indicated by the +. The dashed lines shown the trajectories of the ends of the beams for the model discussed in the text.

separate compact source (such as is often seen in radio galaxies at this frequency) which is blended together with the emission from the western component or it may be part of a bridge connecting the galaxy and the heads of the two components. The 180° symmetry is even more striking if the emission at the position of the galaxy is a separate compact source because then the ridge of extended emission will curve south from the west component in the identical way that the tail curves to the north-east from the eastern component making the symmetry almost perfect about a position RA = 00 h 55 min 42 s, dec = +26°35′44″, 0.5′ east of the galaxy.

Comparison of the 5 GHz map with the 1.4 GHz data indicates a spectral index of −0.7 in the bright inner regions and this steepens to a value of ≲−1.1 in the tail going to the north-east and in the very low brightness region just visible above the noise in the south-west corner of Fig. 1. Other data for this radio galaxy are summarised in the Table 1.

Either component taken separately has a structure remarkably similar to that seen in the tail radio sources but the morphology of the two components together and the location of the galaxy make it impossible to ascribe their shape to a trajectory of the galaxy in this case.

Complex radio source morphologies are now often attributed to the interaction with the intracluster medium, for example, Lari and Perola[12] have shown that in a given luminosity range the number of complex sources found inside rich clusters of galaxies is considerably higher than the number found outside.

Table 1 Observational Data for NGC326—B2 0055+26

Red shift[11]		0.0472
m_{pg}[11]		14.9
Optical position[11] (1950)		RA = 00 h 55 min 40.7 s
		dec = +26°35′44″
Total flux density:	0.4 GHz	4.9 Jy
	0.6 GHz	3.15 Jy
	1.4 GHz	1.7 Jy
	5.0 GHz	0.69 Jy
Angular size		4′
Distance*		141 Mpc
Linear size		160 kpc
Radio power (1.4 GHz)		3×10^{23} W Hz^{-1} ster^{-1}

* $H = 100$ km s^{-1} Mpc^{-1}.

This radio source is not located in a rich cluster and, furthermore, its distortion has a symmetry which cannot readily be explained as an effect of the surrounding medium. Thus we are lead to conclude that in this case the shape is determined by the source energising the radiating plasma rather than by the surrounding medium. The observation of the change in spectral index implies that the regions of high brightness are the youngest so we might consider models in which the energy is supplied by a beam which has rotated through about 90° while generating the source and now points to the region of high brightness. At first sight this kind of model seems somewhat implausible as the region just behind the head is closer to the galaxy so requiring a variation in the power of the beam which is not reflected in the monotonic change of brightness. However, when we consider the effect of projection on the trajectories of the ends of a pair of beams sweeping out two cones on either side of the galaxy (Fig. 2) we can find an impressive agreement with the observations. The dotted lines in Fig. 1 show the trajectories of the ends of the beams in a model in which the beam is offset from the axis of rotation by 18° and wth the axis of rotation inclined to the line of sight by 30°. In such a model the observed change in brightness from the head to the end of the tail is a natural consequence of the increasing age.

Models in which the radiating plasma is energised by a beam emanating from the nucleus of the galaxy have been proposed by Rees[13], Longair *et al.*[14] and Blandford and Rees[15]. Furthermore, Miley[9] and Hendrikson and Bridle (unpublished) have suggested that the S symmetry seen in some sources could be due to the changing orientation of such a beam. For NGC326 the high degree of symmetry and excellent fit to the model indicates a fairly uniform rotation of a beam offset from the rotation axis by a relatively large angle and generating the radiating region at a constant distance from the galaxy. The continual motion of the beam through the intergalactic medium and the maintenance of a constant distance from the galaxy to the radiating region will present some interesting problems and constraints on the types of beam models proposed[13–15].

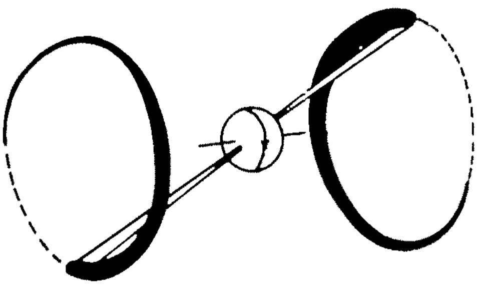

Fig. 2 A sketch showing the essential features of the proposed model.

The rotating offset beam may be caused by a real offset between the beam and the rotation axis of the material supporting it, or the beam may be aligned with the rotation axis of a gaseous disk in the galaxy which is precessing because of a difference between its rotation axis and the principal axis of the galaxy as a whole. The observation of a difference between the rotation axis of the HI and the optical minor axis of the elliptical galaxies NGC4278 (ref. 16) and NGC1052 (ref. 17) provides support for the latter speculation.

We can make a rough estimate of the time scales involved from the observed steepening of the radio spectrum. If this steepening is due to energy losses the age of the ends of the tails would be ≲4×10^{7} yr, however, in tailed radio sources we know that the ages estimated from the kinematics are considerably longer than those determined from the spectral steepening[18,19]. If we compare the steepening of the spectrum seen in this source with that seen in tail sources then the age of the end of the tail is more likely to be ≈2×10^{8} yr corresponding to a period of

~6×10^8 yr for the full circle. In the inner part of elliptical galaxies where solid body rotation is seen the rotation periods are about 10^7 yr (ref. 20), which may be too short to explain the radio data on the basis of the offset beam. If the motion of the beam is caused by precession of a gaseous disk the longer time scale implied by the radio data becomes reasonable.

If the radio axis is associated with a much smaller object such as a black hole in a tilted accretion disk then the motion of the axis will be related to the Bardeen and Petterson effect[21] as discussed by Rees[22].

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Limits on cosmic radio bursts with microsecond time scales

IT has been suggested[1] that black holes of mass $\leq10^{15}$ g evaporate in ~10^{10} yr, ultimately annihilating into a burst of energetic photons and particles. This explosion would produce γ-rays directly, and a radio pulse with a characteristic frequency of ~3 GHz and an energy of ~10^{32} erg may also be generated[1]. It has been shown[2–4] that such a radio burst would be far more easily detected than the corresponding γ-ray burst. Estimates of the energy in the radio burst are uncertain by many orders of magnitude, and it is, of course, not known whether any primordial black holes exist at all; however, the implications of a detection would be enormous for quantum and gravitational physics.

The observational requirements to make an optimal search for a pulse with a ~3 GHz frequency are in themselves interesting as the predicted pulse is only one radio frequency cycle in duration and by definition this event will never be repeated. Such a short pulse will contain all radio frequencies up to a cut-off determined by the actual duration of the pulse so an optimum receiver would have very wide bandwidth, B, and a time constant limited only by the reciprocal of this bandwidth. Unfortunately any pulse originating outside the local region of the galaxy will be dispersed by the interstellar medium thereby reducing the maximum possible sensitivity. For example, in a 10 MHz band at 5 GHz we would expect dispersion to smear pulses to ≥10 μs. It is interesting to note that pulses of this character would not be detected with the time constants commonly used in radio astronomy (even for pulsar surveys) although such a pulse can easily be detected with a suitably designed receiver. The existence of this unexplored time domain for astrophysical phenomena provides additional incentive for such a search.

Limits on the radio pulse emission rate from exploding black holes have already been set by using negative results from wide-beam searches for radio pulses such as might be produced by supernovae[3,4]. In contrast to the small, low-gain antennas used in those searches, we have used the 25-m Dwingeloo radiotelescope; our objective was not only to probe large volumes of space for possible bursts from exploding black holes, but also to observe a variety of specific objects with high sensitivity and time resolution.

Table 1 Sources observed

	Source	Time on-source (h)	Limiting energy (erg)
Planet	Jupiter	2	6×10^6
Star	Algol	3	1×10^{25}
Pulsars	PSR0950+08	3	3×10^{26}
	PSR1133+16	3	3×10^{27}
Supernova remnant	Crab	1	2×10^{30}
X-ray sources	Sco X1	2	3×10^{29}
	Her X1	1	5×10^{30}
	Cyg X1	6	5×10^{30}
	Cyg X2	6	7×10^{28}
Globular clusters	M3	4	3×10^{31}
	M92	6	2×10^{31}
	M15	2	5×10^{31}
Normal galaxy	M31	3	5×10^{35}
Radio galaxy	M87	2	2×10^{37}
Quasar	3C273	3	1×10^{41}

We used a 5 GHz cooled parametric receiver providing a total system temperature of 65 K and an instantaneous bandwidth of 100 MHz. The beamwidth was 10′ arc and the sensitivity was ~0.1 K Jy^{-1}. The detection instrumentation was based on a single channel high speed receiver, which could be optimised for the range of dispersion measures expected. The backend consisted of an input filter of 10 MHz bandwidth with a characteristic double hump shape to facilitate dispersed pulse identification. A detector with a 2 μs time constant followed. Pulses were detected by a comparator which compared an adjustable fraction of the instantaneous power with the average power. Pulses were thus detected when the observed temperature exceeded the average system temperature by a preset factor.

These events were recorded in two ways. Initially, a simple pulse duration discriminator was used to discriminate against pulses which were shorter than expected from dispersion, and the remaining events were indicated on a chart recorder. Later in the observing period it became possible to photograph such detected pulses with the aid of a storage oscilloscope and camera, thereby improving our interference and noise rejection capability.

We observed various objects (Table 1) alternating between a 1 h observation on-source and a 1 h observation at an off-source position 1 h later in RA. The event rate was generally near zero, but occasionally rose to ~10 h^{-1} changing on a timescale unrelated to our observing schedule. Inspection of the events recorded on film showed that the faster event rate was caused by pulses with a regular modulation presumably due to terrestrial interference. Almost every other pulse observed could be attributed either to internally generated spikes (characteristic and repeatable shape, associated with the switching of telescope

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drive motors) or simply to noise (too short for the expected dispersion and consistent with statistical expectations). For the two sources (Jupiter and Algol) for which no dispersion would be anticipated no events of any timescale were recorded. Only one event looked anything like a real dispersed cosmic signal, and that was on a reference for Cyg X-1.

With the discriminator set at about three times the system temperature, the limiting sensitivity is

$$E_{\text{lim}} = 10^{32}\tau\left(\frac{\nu_c}{5}\right)\left(\frac{D}{100}\right)^2 \text{erg} \tag{1}$$

with the pulse duration τ in μs, upper cut-off frequency ν_c in GHz, and distance D in kpc. Dispersion in the interstellar medium determines τ, and for our observing frequency and bandwidth $\tau(\mu s) \approx DM$ (pc cm^{-3}). The dispersion measure (DM) was estimated using a model of the electron distribution in the galaxy[4,5], and substituted in equation (1) to give

$$E_{\text{lim}} \sim 10^{32}\left(\frac{\nu_c}{5}\right)\left(\frac{D}{100}\right)^2 \times\left[\frac{19}{\sin b}(1-\exp(-1.7D\sin b))+10^{-4}D\right] \text{erg} \tag{2}$$

where b is the galactic latitude. The $10^{-4}D$ term allows for additional dispersion by an intergalactic medium with $n_e = 10^{-7}$ cm^{-3}, but this is only important for the most distant sources. Taking the characteristic frequency to equal the observing frequency (the most optimistic case), the limiting energies for the various sources are given in Table 1.

Observations of all sources listed in Table 1 can be used together with the reference observations to set an overall limit on the rate of 10^{32} erg radio bursts from exploding black holes. Taking the maximum distance sampled in each direction as that for which E_{lim} from equation (2) is 10^{32} erg, we find a limit of 9×10^{-6} pc^{-3} yr^{-1}. This compares with the limits derived in the earlier analyses[3,4] of radio data, and is far superior to limits obtained from searches for γ-ray bursts.

It may be more interesting to consider the globular clusters separately from the other sources. If the primordial black holes have a space distribution similar to that of the visible matter in the Universe, then there would be concentrations of them in globular clusters. Taking $10^5 M_{\odot}$ as a typical mass for a globular cluster, the limits in Table 1 imply an upper limit of 10^{-20} yr^{-1} for the fraction of the total mass which evaporates due to 10^{15} g black holes exploding and generating 10^{32} erg radio bursts. In other words, primordial black holes of $<10^{15}$ g can never have exceeded 10^{-10} of the total mass.

An even larger mass is sampled in the M31 observations where our beam corresponds to a linear size of 2.6 kpc. However, the additional dispersion in M31 and the greater distance result in a much higher detection limit.

These limits could be improved by using larger telescopes for longer times and simultaneous observations with more than one antenna. Interference rejection in particular would be greatly facilitated by using two or more widely spaced antennas. Large improvements could be achieved either by using a fast multichannel dedispersing backend or possibly by observing at higher frequencies.

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Photographic amplification of faint astronomical images

METHODS of contrast enhancement in astronomy, such as optical contrasting[1] or optical printing onto a high-contrast material, include all grains throughout the emulsion thickness, and noise (fog), as well as the image, is enhanced. Fairly high levels of chemical fog (up to diffuse densities of 0.3) are common when plates are optimally hypersensitised by baking or prolonged soaking in nitrogen followed by reduction sensitisation in hydrogen gas. Shaw[2] has shown that chemical fog seriously affects the detective quantum efficiency (DQE) of an emulsion when all the fog grains appear in the image as additional noise. I describe here a method that has been devised to make visible extremely faint images on astronomical plates without imaging fog grains. The images are not apparent on visual inspection and cannot be detected by conventional macro-densitometry. These effects result in a significant improvement in the visual detection of faint images on the original plate over the density range occupied by threshold imagery. The technique is most useful for revealing faint nebulosity and low surface brightness features on fine-grain high-contrast emulsions such as Kodak spectroscopic plates type IIIa.

The technique depends on the fact that faint images tend to be located in the uppermost layers of the developed emulsion whereas grains due to chemical fog are distributed at random throughout the total thickness of the emulsion coating. Unlike most film products, spectroscopic plates do not have a transparent protective super-coat to prevent abrasion. The faintest and sharpest images therefore occur at the emulsion–air interface. By using a diffuse-light vacuum-contact printer it is possible to make contact copies of original plates which record the upper layer image only. Fog grains at lower levels are not resolved and add a uniform neutral density which has no effect on the image. The signal-to-noise ratio is thus improved. With sky-limited plates the faint signal is only slightly denser than the uniform exposure from the sky background. The use of a high contrast copy film and careful control of exposure ensure that a very narrow range of densities around the signal level is expanded without the addition of background noise from deeper within the emulsion.

To produce the contact copies a commercially available diffuse-light vacuum-contact printer is used. A simple modification to enable a wide range of original plate densities to be handled has been previously described[3]. Agfa Gevaert graphic arts film type FO 71p has several characteristics which make it particularly suitable for use with this method. This high-contrast film has an orthochromatic fine-grain emulsion with developer components built into it for use in a stabilisation processing machine. The film can be processed in any alkaline solution but in practice a standard print developer at normal strength gives rapid and uniform results by processing in open trays. Development is effectively complete in less than 2 min at 20°C and consistent results are not significantly dependent on the degree of agitation in the developer solution. Conventional stop bath, fixation and washing complete the processing. Lithographic (infectious development) materials produce too much contrast for this method.

The contact exposure is adjusted so that the true sky background, well away from any nebulosity or vignetting, develops to a diffuse density of 0.4–0.6 on the copy positive. At this point on its characteristic curve the film has a very high gamma, so exposure is critical. The processed copy may be enlarged or contact printed onto a hard grade of paper to give a negative print. Again, exposure is critical and should be arranged so that substantial density is produced in the sky background.

The method is most successful with fine-grain high-contrast plates such as Kodak types IIIaJ and IIIaF. In coarser-grained emulsions faint images are scattered to greater depths within the recording layer[4] and the contrast in faster emulsions tends to be lower. Copies of plates are less suitable than originals as the relative positions of image and fog grains have been changed, particularly if a collimated or distant point light source was used to make the first copy.

Unlike most optical methods of copying, the technique can be used with plates of any size, limited by the contact printer and available film stock. The largest plates copied to date have been 355×355 mm plates from the UK 1.2-m Schmidt telescope. Dish processing of these large sheets of film is possible because the process is so simple. However, the originals must be of the best quality. Plates taken in very thin haze which appear perfectly acceptable show alarmingly large haloes around the brighter stars when copied as described. The extent of telescope vignetting and internal reflections are likewise clearly revealed as are development defects and marks due to handling and surface abrasions on the original plates.

IIIa plates exposed so that the sky background density reaches about 1.0 (ANSI diffuse) above fog seem to show the faintest objects. In these circumstances all the faint imagery is on the steep part of the characteristic curve where the output signal-to-noise of the emulsion is at a maximum[5]. Figure 1 is a high-contrast derivative of a sky-limited IIIaJ/GG 395 plate of Centaurus A (NGC5128) taken on the UK 1.2-m Schmidt telescope at Siding Spring, Australia. The visual appearance of the original plate is illustrated by the inset, which has the same scale and orientation. Both prints were obtained from the same plate. The NE–SW extension and western loop of Cen A described by Johnson[6] are almost burned out in this reproduction but much new filamentary material can be seen, particularly to the south and west. The reality of this faint material is confirmed on other high-contrast derivatives made from different original plates; it continues on to adjoining SRC Sky Survey fields. It could well be galactic in origin and not associated with Cen A. Even so, that nebulosity which is obviously associated with Cen A extends along the major axis over a distance of approximately 1.2°.

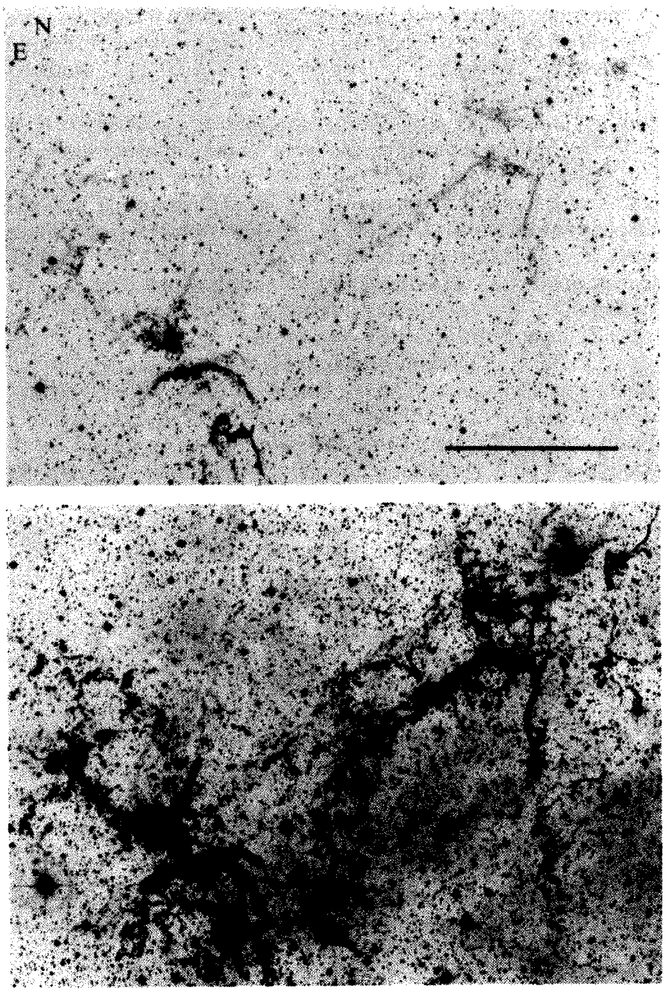

Fig. 1 Faint filaments around Cen A (centre). Inset shows visual appearance of original plate. Both prints have the same scale and orientation and are from the same IIIaJ plate taken on the 1.2-m UK Schmidt telescope. Scale bar, 30 arc min.

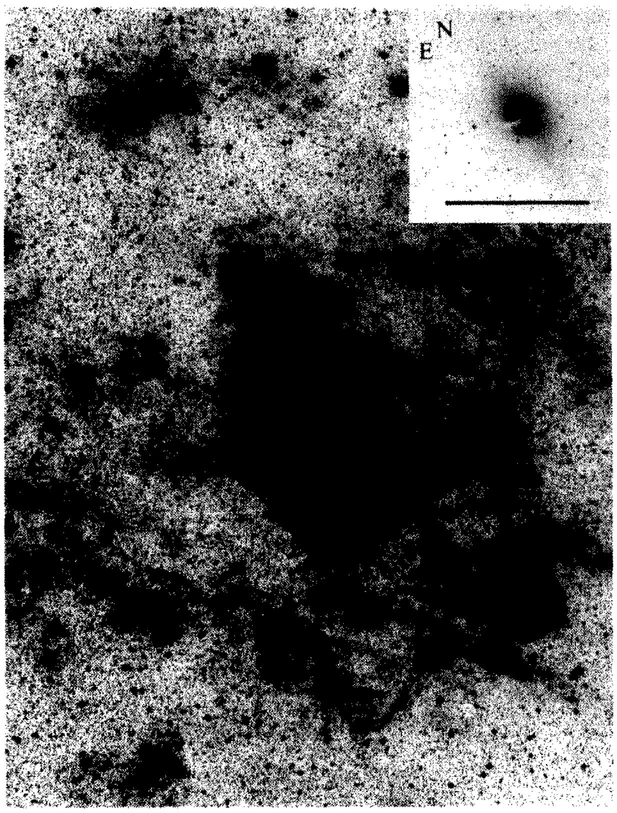

Fig. 2 Part of the Puppis supernova remnant from a deep IIIaF/RG630 plate taken on the 3.9-m Anglo-Australian telescope. A normal copy of the plate (above) is compared with a contrast-enhanced version of the same plate (below) printed with the same scale and orientation. Scale bar, 5 arc min.

The quantitative brightness of the faintest objects revealed by the technique is difficult to determine. However, the method seems capable of resolving structure about 0.5% brighter than the night sky (Cannon, personal communication), and Carter (unpublished results) has recently derived a B value, from deep IIIaJ plates of 22.9 mag arc s^{-2} for the night sky at Siding Spring. These values imply that the faintest features on the prints have a surface brightness of about 29 mag arc s^{-2}.

Figure 2 shows part of the Puppis supernova remnant, photographed on a IIIaF/RG630 plate using the 3.9-m Anglo-Australian telescope. Both illustrations have the same scale and orientation and are made from the same plate. The upper print represents the normal visual appearance of the plate but the lower print, made with the amplification technique, shows faint filaments and diffuse background nebulosity not previously observed.

This simple technique has been used with success on objective prism plates, direct and image tube spectra plates and non-limiting exposures on spectroscopic plates from several sources. It can be undertaken with commercially available equipment and materials and requires no special skill in its operation.

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Possible identification of the 45-μm ice signature in Orion

THE composition and size distribution of interstellar dust are controversial matters. Even the ice contribution to the well known and conspicuous 3-μm feature of the extinction curve has recently been questioned. We now report a study of the middle-infrared spectrum of dust emission. This spectral region is of interest because it includes the 45-μm band of water ice which, unlike the 3-μm feature, is characteristic of the ice lattice—being essentially due to stretching vibrations of the H–O bond. The 45-μm band also carries information on the physical state and temperature of the ice[1]. If the ice is amorphous, the band is wide (~19 μm) and, at 100 K, peaks at 45 μm; crystalline ice produces a narrow peak ~2 μm wide at 43.5 μm, for a temperature of 100 K; these characteristics are all temperature dependent. Note that the only possible crystalline phase at pressures below 1 bar is Ic (cubic) or Ih (hexagonal), for both of which the above statements are true. The relevant lattice vibrations are essentially translational and probably associated with the transverse optical branch of the dispersion diagram. Our results suggest an emission due to predominantly amorphous ice.

In the simplest astrophysical model, the 45-μm feature can be observed in emission from hot sources or in absorption through cold dust against a hot background. In the Northern Hemisphere, the bright Kleinmann-Low (KL) nebula is particularly suitable[2]. Observations were made using a grating spectrometer mounted at the Cassegrain focus of a 30-cm telescope on board the C.E.V. Caravelle, with a focal distance of 2,400 mm (ref. 3). Other system characteristics are given in Fig. 1 legend.

The airborne observations were carried out over France on 28 February and 13 March 1978, at a constant altitude of 11,300 m. The outside temperature was −53 °C and the dew point −55 °C. The pressure was ~210 mbar and the line-of-sight elevation remained between 27 and 33 °C. The telescope was pointed to the KL nebula, offsetting from θ^1C in the Trapezium, and the field of view was 4.5 and 1.8 arc min in elevation and azimuth respectively. The latter corresponds to a spectral slit width of 2.5 μm for an extended source and 1.3 μm for a point source, if alignment is perfect.

The overall spectral transmission (t_i) of the instrument, including the detector, was calibrated against a black body in the laboratory, before and after the flight. Atmospheric absorption bands were used to calibrate the wavelength scale. From the difference between spectral transmissions obtained at black-body temperatures of 200 and 400 °C, we conclude that the error on relative transmission values is <10%. The error on absolute values is not relevant to our purposes. Atmospheric transmission (t_{atm}) was computed on the basis of a standard Earth atmosphere[4] for different moisture contents (w) and spectral slit widths $(\delta\lambda)$. A satisfactory fit to the observed spectrum is obtained for 5.2 μm zenithal precipitable water and $\delta\lambda = 1.5$ μm. The average (I_{raw}) of eight of the recorded spectra, taken in identical conditions (except for the interchange of beams, which eliminates spurious offset signals) was considered as representative of the data, and divided by $t_{atm} \times t_i$ to give the spectrum (I_{KL}) of the nebula.

In addition to the usual noise sources, our study was affected by fluctuations in the effective wavelength of the detected radiation for a given setting of the grating; this was due to telescope pointing fluctuations. Using the apparently fluctuating locations of the H_2O absorption bands at 35.6, 40.2 and 44.3 μm, the standard deviation (σ) was ~0.4 μm (corresponding to a pointing deviation of 0.7 arc min). As a consequence, averaging over several spectra leads to an apparent widening of the slit, which was taken into account in computing t_{atm}.

Figure 1 represents the spectrum of the KL nebula, I_{KL}, together with that, I_b, of a body at 80 K having an emissivity proportional to λ^{-1}. The latter is very close to the spectrum of a grey body at 100 K and both fit reasonably well experimental data obtained at lower resolution over a wider band[2,3]. Our data are similar to those obtained by Ward[5] in the same range. Both studies indicate a bump at about 43 μm. If this is attributed to the amorphous ice feature, with a peak at $\lambda_0 = 43$ μm and a FWHM $\delta\lambda = 19$ μm, then, trying to fit a function of the form

$$I_b\left\{A + B\exp\left[-4\ln 2\left(\frac{\lambda - \lambda_0}{\delta\lambda}\right)^2\right]\right\}$$

through the data points gives $A \simeq 1$, $B \simeq 0.4$, corresponding to the dashed curve in Fig. 1. This set of values is obtained assuming a constant r.m.s. noise voltage over the spectral range and the validity of the fit is confirmed by the χ^2-test (reduced $\chi^2 \simeq 1$). The error bar at 43 μm in Fig. 1 represents the relative standard deviation of the fit $(\pm\sigma/A)$. (At 43 μm, this is equivalent to a r.m.s. noise voltage of 120 nV Hz$^{-1/2}$ on the detector, per spectral element). The choice of the exponential form for fitting was made for lack of a better knowledge of the ice 45 μm band profile in terms of dust extinction. It was intended to assess the objective existence of a feature near

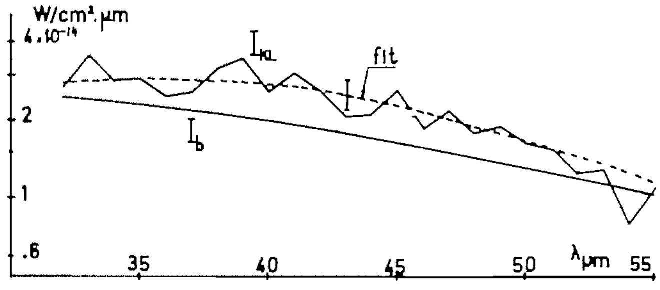

Fig. 1 I_{KL}, average measured spectrum of KL nebula, corrected for instrumental and atmospheric transmission. I_b, grey body spectrum, $T = 80$ K, $\varepsilon = \dfrac{30}{\lambda\,(\mu m)} \times 0.1$. Dashed curve, least squares fit to I_{KL}. Error bar $\pm\sigma/A$, where σ is the standard deviation of the fit (see text). Spectrometer system characteristics: aluminium echelette grating, ruled at 20 lines per mm, 20×20×6 mm in size blazed at 42 μm, in a folded Czerny–Turner design. The entrance hole is circular, 3.2 mm diameter. Average system transmission 3%; detector is a Low bolometer, with BaF_2 Reststrahlen filters. The grating is driven by a synchronous motor, through a gearbox at an average speed of 0.1 μm s^{-1} between 30 and 60 μm. The telescope secondary is wobbled at 20 Hz, with a sinusoïdal beam throw of 3 arc min.

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45 μm, which it does indeed, since B/A (40%) is clearly larger than σ/A (17%), notwithstanding possible errors in instrumental spectral response (<10%).

The data therefore seem to indicate the presence of an emission due to predominantly amorphous ice. Its relative amplitude (B/A) is, however, much smaller than predicted for an optically thin cloud permeated with 'standard' core-mantle grains[6,7]. Since KL is indeed thin in the wavelength range considered ($\tau \sim 0.1$, from the comparison of colour and brightness temperature), the data would point to a shell model where the ice mantles are only present at the periphery of the nebula[6]. A satisfactory fit of such a model to the experimental data still requires proper tailoring of grain dimensions and densities, shell radii and central heating source.

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Atomic oxygen emission at 63 μm as a cooling mechanism in the thermosphere and ionosphere

FAR IR emission by atomic oxygen fine structure transitions is considered an important cooling mechanism in the Earth's upper atmosphere and ionosphere as well as in several astronomical objects. The cooling rates so far have only been calculated theoretically on the basis of local thermodynamic equilibrium. No experimental or theoretical determination of the fine structure excitation or quenching cross sections for mutual neutral atomic oxygen collisions has been reported. We report here the first spectrometrically measured atomic oxygen 63-μm emission profile at thermospheric altitudes (90–180 km). An energy-averaged quenching cross section for the $O(^3P_1)$ level was deduced. The importance of the 63-μm emission as a cooling process for both the neutral thermosphere and the ionosphere is much less than predicted.

On 16 March 1977, at 2304 CET a liquid helium cooled far IR grating spectrometer was launched aboard a Skylark 7 rocket from Andoya, Norway, as part of the payload AI/II-PHA-F4D of the 'Polar High Atmosphere' rocket campaign (see ref. 1 for details). During the flight the geomagnetic field was moderately disturbed (200 nT). Measurements were taken in the wavelength interval of 45–125 μm.

Figure 1 shows preliminary absolute zenith intensities of the 63-μm line measured by this experiment (code name AB3). The data are compared with a model calculation by Kockarts and Peetermans[2] (KP1 and KP2). This model includes radiative transfer and is valid under the following assumptions: (1) the atomic oxygen is in diffusive equilibrium; (2) the fine structure level occupation densities obey a Boltzmann distribution at the local gas kinetic temperature, that is, local thermodynamic equilibrium (LTE) is assumed; (3) no additional non-thermal excitation processes are accounted for. Two curves from this model are shown in Fig. 1: curve KP1 assumes an atomic oxygen number density of $1\times10^{11}\ \text{cm}^{-3}$ at 120 km; curve KP2 is valid for a corresponding value of $5\times10^{10}\ \text{cm}^{-3}$. The exospheric temperature is 750 K for both curves, close to the exospheric temperature of 800 K at the time of the flight. At low altitudes the 63-μm line becomes optically thick. The agreement between the measurement and the model curve is quite good at these altitudes, taking into account experimental error (bar) and approximations used for the model calculation. At higher altitudes, however, the measured intensities are much lower and exhibit a much steeper altitude gradient than the calculated ones. Neutral gas densities, including atomic oxygen, were measured by a mass spectrometer during the upleg of the rocket trajectory (E. Hettmannsperger, P. Lämmerzahl and D. Krankowsky, personal communication). The slope of the measured atomic oxygen density profile does not show any substantial departure from a diffusive equilibrium density profile for a 800-K exospheric temperature. Despite local and temporal differences between the upleg and downleg trajectory, the model assumption of diffusive equilibrium seems satisfactory in the conditions of this experiment.

The steep gradient of the measured 63-μm line intensities can be understood if the atomic oxygen line is not in LTE. The atomic oxygen concentration in the ^3P_J state may then be described by

$$n_J = n(\text{O})\frac{f_J g_J \exp(-E_J/kT)}{\sum f_J g_J \exp(-E_J/kT)}\quad J=2,1,0 \qquad (1)$$

where $n(\text{O})$ is the total atomic oxygen number density, g_J the statistical weights $2J+1$ of level J, E_J the energy above the P_2 ground level, k Boltzmann's constant, T the local gas kinetic temperature, and f_J the deviation from a Boltzmann distribution. The functions f_J are determined as a function of altitude by solving the steady-state equation for the three fine structure level densities. The steady-state equation includes spontaneous and induced emission, reabsorption of radiation and collisional activation and quenching. A first approximation of the solution is obtained by neglecting any effects caused by collisional and radiative transitions to and from the $^3P_0$ level.

Two curves, NB1 and NB2 in Fig. 1, were calculated for a non-LTE distribution of the fine structure level densities. Curve NB1 was derived from the density and temperature conditions of the KP1 profile. Curve NB2 is valid for the corresponding conditions of the KP2 profile. The specific radiation intensity needed for solving the steady-state equation was derived from the measured 63-μm intensity in the optically thick altitude regime and was assumed to be altitude independent. Both curves fit the measured altitude gradient reasonably well. An energy-averaged quenching cross section of about $10^{-20}\ \text{cm}^2$ is adopted to produce these curves. Best agreement with the measured intensities is obtained with the profile NB1. Increasing the quenching cross section by a factor of 2 already causes a considerable discrepancy between measured and calculated profile gradients. Still lower cross sections are possible, however, as the curve approaches a radiation equilibrium profile. The value of $10^{-20}\ \text{cm}^2$ is therefore an upper limit at this early state of the data analysis.

Atomic oxygen fine structure levels may also be excited by electron impact. This process is thought to act as an important cooling mechanism for ionospheric electrons[3]. In this case electron densities and temperatures were measured simultaneously on the same payload (A. Dumbs and E. Neske, personal communication); atomic oxygen excitation by electrons was therefore included in a second step in the steady-state equation. The electron energy loss rate given by Dalgarno[4] was used and divided by a factor of 2 following a suggestion by Hoegy[5]. The 63-μm line intensities calculated for thermal and electron impact excitation in non-LTE conditions based on the profile NB1 are shown in Fig. 1 (NE1). The difference between the values of the curve NE1 and NB1 shows the effect of the additional electron excitation. It seems that the cross sections for electron impact excitation of atomic oxygen fine structure levels by Hoegy[5] are still too high by a considerable factor. The detailed evaluation of the interplay between thermal and electron excitation of the 63-μm line is now underway.

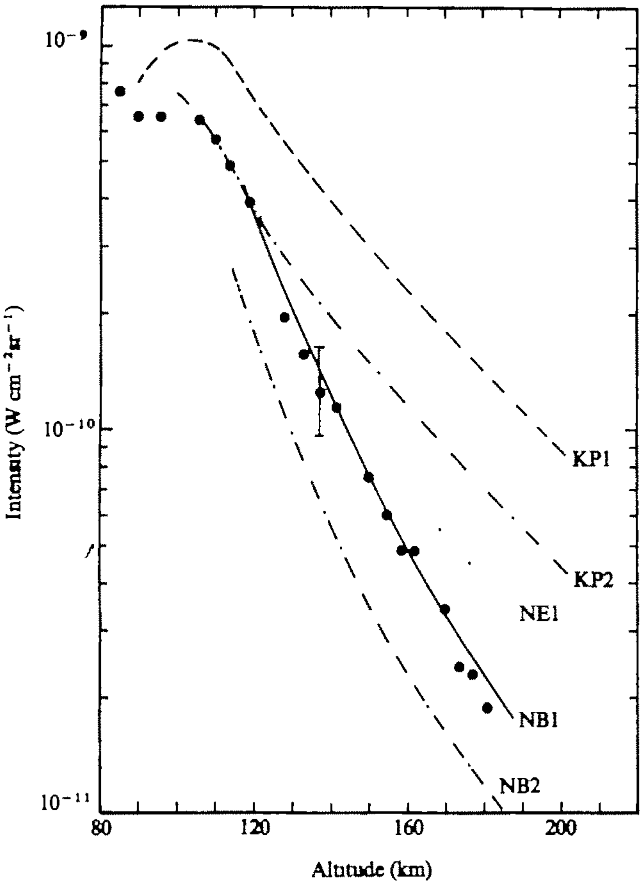

Fig. 1 Atomic oxygen 63-μm zenith intensities. ●, Individual data obtained during downleg of rocket trajectory; other labels, see text.

The preliminary 63-μm emission profile reported here has considerably lower emission rates than predicted and the importance of the 63-μm emission as a cooling process for the neutral thermosphere is much less than previously assumed. Measurements have enabled the determination of at least an upper limit of the fine structure quenching cross section for O–O collisions and the deduced value of 10^{-20} cm^2 is several orders of magnitude less than was previously assumed. The altitude dependence of the electron cooling rates as found experimentally[6] does not agree at all altitudes with the theoretical expectations for the energy transfer from electrons to neutral atomic oxygen directly or by ions. Lower 63-μm emission rates and lower electron to atomic oxygen excitation cross sections as described here would help to lessen this discrepancy.

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Helium gas bubble lattices in face-centred-cubic metals

ION irradiation of metals with helium or hydrogen isotopes commonly results in the formation of microscopic gas bubbles. At very high doses, radiation blistering and related phenomena can cause severe damage to surfaces[1]. Much research has been stimulated by the need to find materials with acceptable erosion and gas loading properties for use in future controlled thermonuclear reactors. Bubble formation is also important in fission-reactor fuel technology and other applications[2,3]. The underlying bubble structure of face-centred-cubic (f.c.c.) metals has remained largely unknown, although much has been done on depth profiling light gases implanted to high doses (see, for example, refs 3–5). Inert-gas bubbles lying on a space lattice have been reported previously only for the body-centred-cubic (b.c.c.) metal molybdenum[6,7]. There have been many observations, mainly for b.c.c. metals, of the similar ordering of voids produced by particle irradiation[8,9]. The void lattice spacing is typically 5–10 times that found for bubbles (~5 nm). We report here on the bubbles produced in several f.c.c. metals by 30 keV helium-ion irradiation to a level just below the critical dose for blistering. A new result for this important class of metals is that the helium gas bubbles lie on a superlattice having an f.c.c. structure with principal axes aligned with those of the matrix.

Annealed polycrystalline targets electropolished to a high surface finish were irradiated at room temperature to a level of approximately 4×10^{17} He ions cm^{-2} with the ion beam normal to the surface. The main study was of copper, for which the mean projected range and r.m.s. spread in projected range, calculated using the Harwell version[10] of the computer code E-DEP-1 of Manning and Mueller, are 97 nm and 43 nm respectively. To ensure that the samples were all at approximately the same stage of bubble development, an ion beam having a lateral gradation in current density was used. Radiation blisters formed first where the current density was highest[3] and the region of the target which was close to blistering could be easily identified. Samples were taken from this area and thin sections containing the bubble layer prepared for transmission electron microscopy.

Bubbles in copper, typical of those observed for helium-ion irradiation at 300 K, are shown in Fig. 1. Considerable bubble alignment can be seen with dense-packed rows parallel to matrix {111} trace directions. Diffraction from the bubble array caused the splitting evident around the matrix reflections in the electron diffraction pattern of Fig. 2. The strongest reflections in the superlattice pattern are assigned (111) indices as they are in line with the (111) matrix spots and are in the positions expected for diffraction from the dense-packed planes seen edge-on as bubble rows in Fig. 1. The results show that the bubble-lattice is at least predominantly an f.c.c. structure aligned with the copper matrix. The bubble diffraction patterns for the matrix orientations [100], [111] and [112] support this conclusion. Alignment conforms, as expected, to crystallographic features in the

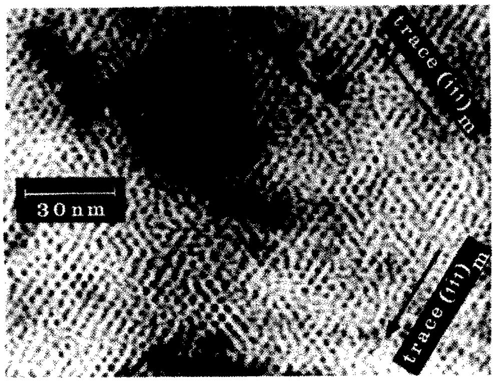

Fig. 1 Direct transmission electron micrograph of helium gas bubbles in copper following helium-ion irradiation to a level of $\sim 4\times10^{17}$ 30keV He ions cm^{-2} at 300 K. The electron beam is along [110] in the matrix and is normal to the plane of the bubble layer. The directions of the traces of the (111) matrix planes in the (110) plane are indicated. The bubble lattice constant, a_l, found from 380 measurements of inter-bubble spacing is 7.7 ± 0.8 nm.

copper; bubble rows run parallel to matrix slip traces and assume the twin-direction on crossing twin boundaries.

The lattice constant found, $a_l = 7.6\pm0.3$ nm, corresponds to approximately 10^{19} bubbles cm^{-3}. There is good agreement between the results both for samples from the same irradiation and for the several different copper targets used. The lattice constant based on diffraction from a given set of planes is consistent for the above orientations. There are, however, small but apparently significant differences between the different planes. For example, the {200} planes give a consistent value for a_l of 6.8 ± 0.4 nm. Some variations in structure are apparent and will be reported eslewhere. Direct micrographs at [110] show domains of good ordering typically of five inter-bubble spacings across with regions between of comparable size in which the ordering is less obvious.

The average bubble diameter found using a through-focus technique is 2.0 ± 0.5 nm. The ratio of lattice spacing to bubble radius is in the range of values found for void lattices[9]. This suggests there may be a common mechanism of lattice formation. If all the implanted helium is contained in bubbles of the size and separation measured here, then it can be estimated that the bubbles may be over-pressured by a factor of 5 or more. The difficulty is in relating helium pressure to atomic density at very high densities[11]. Over-pressure is a necessary element in a recent theory of blister formation[11]. The associated volume swelling is 5%; the swelling could be higher and the degree of over-pressure less if there were an additional large population of small gas bubbles below the resolution limit of the microscope (~1.5 nm for cavities in copper). We have been unable to detect this either in diffraction at 300 K or in direct microscopy on specimens heated to induce bubble growth. For this reason the existence of a significant population is thought to be unlikely. The bubble lattice seems to form only at high dose ($\gtrsim 2\times10^{17}$ He ions cm^{-2}), but once formed it is stable at temperatures up to approximately $0.3T_m$. Following blistering at 300 K, a bubble lattice with similar parameters has been seen here to survive in some blister caps as well as in the region between blisters.

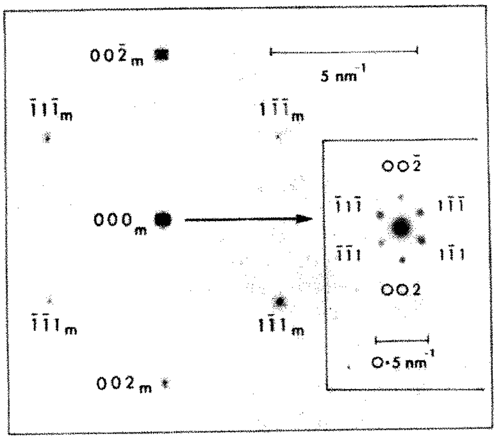

Fig. 2 A selected-area electron diffraction pattern from the specimen of Fig. 1. The electron energy is 100 keV. The matrix pattern is shown on the left and the superlattice diffraction pattern centred on the (000) transmitted beam is shown enlarged in the inset. In the latter slight changes in specimen tilt and translation have been made to obtain more uniform excitation. The value of a_l deduced from the separation of the (111) spots is 7.6 ± 0.3 nm.

Similar helium-bubble lattices are seen after irradiation at 300 K in the two other f.c.c. metals studied, nickel and AISI 321 (18Cr : 10Ni : Fe) austenitic stainless steel. The lattice constants based on diffraction from (111) superlattice planes are 6.6 ± 0.5 nm and 6.4 ± 0.5 nm respectively. At 300 K copper is above the vacancy mobility temperature while nickel and stainless steel are below. As in molybdenum, vacancy mobility does not seem to be a major factor in forming the lattice, at least in the limited range of low mobility covered by these metals. In this context it is interesting that considerable bubble alignment has been observed in the present experiments in proton-irradiated copper where the rate of vacancy production by the primary beam is a factor of 10 less than for helium-ion irradiation.

The reproducibility of the results suggests that bubble lattice formation could be a common feature of high-dose inert-gas implantation in annealed f.c.c. metals at low temperature. P.B.J. thanks The Nuffield Foundation for financial assistance.

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Crown ether intercalations with phyllosilicates

IT has been discovered in the past decade that some macrocyclic polyethers (crown ethers) act as ligands to form stable complexes with salts of numerous metals. These are the first synthetic compounds able to coordinate to alkaline cations[1-3].

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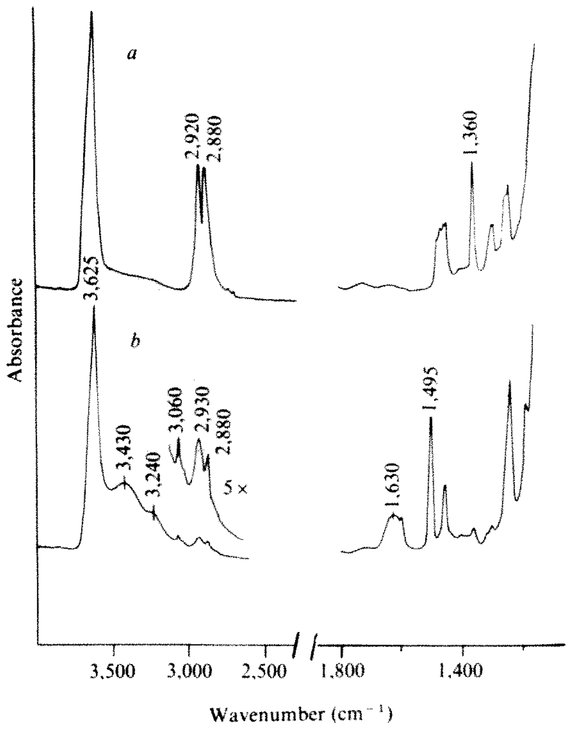
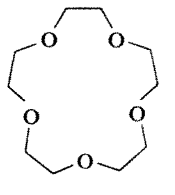
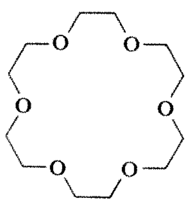


Fig. 1 Infrared spectra of Ba-montmorillonite treated with a methanolic solution of: *a*, 15-crown-5; *b*, dibenzo 18-crown-6.

The cyclic structure of the crown ethers creates a circular cavity of oxygen atoms to which the cations coordinate. The diameter of this cavity as well as the relative size of the cation, determine the stability of the resulting complexes, and the cationic selectivity depends on it. The most studied crown ethers are derived from so called 15-crown-5 and 18-crown-6:



15-crown-5



18-crown-6

We have shown experimentally and report here that the crown ethers are able to intercalate between the layers of the 2:1 phyllosilicates, saturated with alkaline or alkaline-earth cations, showing an unusual stability. The complexes were obtained mainly by immersion of homoionic montmorillonites (Wyoming bentonite), in different solutions of crown ethers. Hectorite and vermiculite were also tested with positive results.

Many neutral organic substances, such as alcohols, ketones, phenols, and aromatic hydrocarbons, can intercalate the interlamellar space of clays of the smectites group[4-5]. Generally, the fixation of these compounds is due to their interaction with the exchangeable cations, which balance the deficit of charge of the silicate layer. Nevertheless, the stability of those complexes is relatively low, especially in the presence of alkaline cations. Water molecules also easily replace the organic ligands, by exposition to atmospheric moisture.

The coordination between the interlamellar cation and the crown ether, can be shown by the UV spectra of montmorillonite treated by dibenzo-18-crown-6. The band at 275 nm, due to the aromatic ring, is displaced in the Na-, K-, and Ba-montmorillonite complex, to 285 nm. A shift of 6 nm has been observed in complexes of the same crown ethers with different inorganic salt solutions. The shift was explained by the bathocromic effect of the cation associated to the aromatic ring[1].

Table 1 Values of the increase in the basal spacings (Δd) in homoionic montmorillonites (M–K^+, M–Na^+, M–Ba^{++}) treated by some crown ethers.

Crown ethers	Δd_{001} (Å) M–Na^+	 M–K^+	 M–Ba^{++}
12-crown-4	8.1	4.4	6.6
15-crown-5	4.0	4.4	8.0
18-crown-6	6.3	4.4	3.9
dibenzo-18-crown-6	8.9	7.9	7.7
dibenzo-24-crown-8	8.2	9.0	7.1

The basal spacing (d_{001}) of the anhydrous montmorillonite is 9.6 Å.

The IR spectra show that the coordination of the crown ether with the interlamellar cation produces a displacement of water molecules which originally belonged to the sphere of coordination of the cation. Figure 1*a* shows the IR spectrum of the Ba-montmorillonite complex 15-crown-5 where the H_2O bands have disappeared. However, when crown ethers of a larger size are intercalated, the steric effect prevents the complete association to the cations, and the water molecules are not completely displaced. Figure 1*b* shows the spectrum of Ba-montmorillonite/dibenzo 18-crown-6 complexes, in which the bands at 3,430 and 3,240 cm^{-1}, ν(OH) stretching, and the 1,630 cm^{-1}, δ(HOH) bending are visible but less intense. The displacement of water molecules is irreversible, in contrast with the already mentioned complexes with other neutral organic molecules.

The intercalation of the crown ethers in phyllosilicates can be also followed by X-ray diffraction. In most cases the increase of the basal spacing are either 4 or 8 Å (Table 1), which may be interpreted as monolayer or two-layer complexes respectively. The macrocyclic ligands are arranged parallel with respect to the silicate layers, in agreement with the thickness of the crown molecules. The 001 reflections present integral orders, suggesting a reasonable crystalline order for most of the montmorillonite complexes such as 15-crown-5,18-crown-6 and its derivatives. In some cases interstratifications are obtained.

The high stability of these new complexes is the most interesting feature: the crown ether is not desorbed by water treatment, organic solvents or salt solutions. In salt solutions the complexes can maintain the cation exchange capacity of the original mineral. Besides, thermal treatment is not able to desorb the crown ethers, as shown by the exothermic peak around 350 °C, observed by DTA.

Note added in proof: Intercalation complexes of phyllosilicates saturated with alkaline, alkaline-earth or transition metal cations have been recently prepared in our laboratory with polyoxadiazamacrobicyclic ligands ('cryptands').

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Measurement of thermal maturation of petroleum by proton magnetic resonance spectroscopy

THE processes of petroleum generation and maturation have been shown to depend on the thermal breakdown of organic material contained in the sediments as the temperature increases due to increased burial depth[1,2]. Measurement of the degree of thermal alteration of the organic matter in shales and other potential petroleum source rocks is therefore of fundamental importance to petroleum exploration. The most common methods of estimating the degree of thermal alteration are based on measurements of the residual insoluble organic material in rocks. Some of the more common methods use changes in the chemical composition of the kerogen[2], electron spin resonance[3], kerogen coloration[4] and vitrinite reflectance[5]. The method reported here is based on measurements on the soluble aromatic hydrocarbons extracted from the sediments. As the aromatics are represented in both the source rock and reservoir petroleum they may eventually provide a useful medium for comparing the thermal history of reservoir and source rock hydrocarbons. This method uses well established chemical techniques and provides a simple quantitative measure of thermal maturation by measuring the percentage of aromatic protons in the aromatic fraction of petroleum.

Samples of petroleum-bearing shales and siltstones were obtained from drilling operations in the Carnarvon Basin of Western Australia. Samples from different locations and depths of a uniform sequence of Cretaceous marine sediments were studied. Geochemical data for the samples are shown in Table 1. Each sample was crushed and the soluble organic material was extracted with methylene chloride : methanol (20 : 1) under the influence of ultrasonic vibration. After careful distillation of the solvent at atmospheric pressure the residue was chromatographed on silicic acid. Consecutive fractions were eluted from the column with pentane, ether and methanol. The fraction eluted with ether was retained as aromatic hydrocarbons and analysed by proton magnetic resonance spectroscopy.

The signal from the aromatic protons (δ 6.7–7.9) was integrated and expressed as a percentage of the total proton integral. This value, the percentage of aromatic protons in the aromatic fraction, will be referred to as the PAP value of an oil or extract.

Although thermal evolution of organic matter depends on both time and temperature, the time factor has been taken as constant for all samples and the PAP values have been plotted against the present sediment temperatures (Fig. 1). The assumption that each set of samples has a similar thermal history is supported by geological evidence that subsidence occurred at each location in the basin over approximately the same time interval, and that the sediments are now at their maximum depth of burial[6]. Samples from three different wells over a temperature range of 60 °C show a regular increase in their PAP value as the temperature increases. This correlation of temperature and PAP value is attributed to the effect of thermal processes on the petroleum and is supported by pyrolysis studies of low maturity hydrocarbons. Figure 2 shows PAP values plotted against time for petroleum which had been subjected to pyrolysis at 300 °C for various times. In these experiments, the low maturity petroleum was contained in a matrix of shale and pyrolysed in evacuated glass tubes. Blank experiments, without added petroleum, showed that the contribution from pyrolysis of hydrocarbons already present in the shale was negligible.

These experiments showed that the changes in the PAP value which result from pyrolysis of an immature petroleum are similar to those which are observed in nature as the sediment temperature increases. It is therefore reasonable to conclude that the variation in PAP value of sediment extracts reflects the level of maturity of organic material in the sediment.

Vitrinite reflectance data for one set of samples are shown in Table 1. Both the PAP and vitrinite reflectance values are consistent and indicate that the organic material has undergone

Table 1 Geochemical data

Sample	Depth (m)	Sediment temperature (°C)	PAP	Vitrinite reflectance (%)	CPI (C_{23}–C_{29})
BH 1.1	1900	73	13.3	0.63	1.22
BH 1.2	2100	78	13.7	0.72	1.48
BH 1.3	2300	85	15.6	0.82	1.17
BH 1.4	2400	89	16.5	0.86	1.20
BH 1.5	2600	99	18.8	0.95	1.23
BS 1.1	1600	62	8.5	—	0.86
BS 1.2	2000	76	12.0	—	1.34
BS 1.3	2300	86	14.9	—	1.24
BS 1.4	2500	92	17.9	—	1.18
BW 2.1	2260	84	14.4	—	0.96
BW 2.2	2440	92	16.0	—	1.01
BW 2.3	2640	99	17.4	—	0.94
BW 2.4	2840	108	19.8	—	0.99
BW 2.5	3140	120	21.9	—	1.02

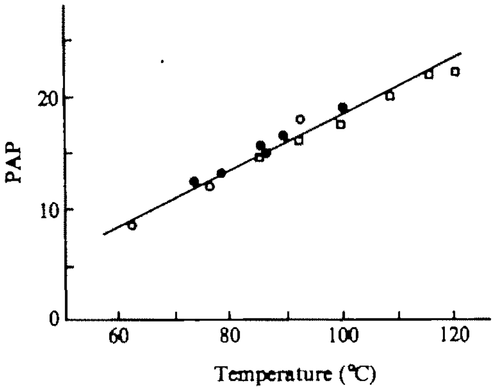
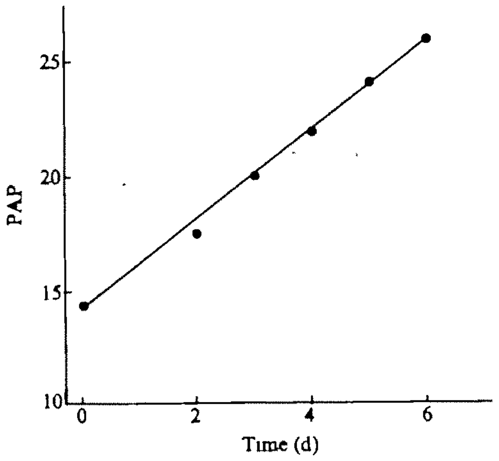


Fig. 1 PAP values plotted against the sediment temperature. Sets of samples from different wells are indicated. ●, Well BH; ○, well BS; □, well BW.

Fig. 2 PAP values plotted against time for pyrolysis of an immature petroleum on shale at 300 °C.



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moderate levels of thermal maturation. The samples investigated differed in their CPI values and in the proportions of *n*-alkanes and napthenes. This suggests that this new technique is useful for samples containing a range of chemical types; however, further work is required to fully assess the validity of the technique for a greater range of kerogen types.

This technique may be a useful supplement to existing methods of measuring thermal maturation as it provides a sensitive measure over the small but important temperature range where liquid petroleum is generated and matures.

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Oxidation state of plutonium in the Irish Sea

SEVERAL of the chemical parameters needed to understand the distribution and behaviour of plutonium in the aquatic environment have not been fully investigated in the field. We consider here two of the more fundamental of these parameters: (1) the oxidation state(s) of the plutonium present in seawater; and (2) the extent to which each oxidation state is associated with suspended particulate matter. A widely accepted analytical technique is adapted to separate the two oxidation states expected to show a high affinity for particulate matter, Pu(III) and Pu(IV), from the more soluble pair, Pu(V) and Pu(VI), and is applied to a series of seawater samples. It was found that in the area studied, generally >75% of the plutonium in solution, and <10% of the plutonium on particulate matter, was Pu(V+VI); consequently the distribution coefficients for Pu(III+IV) exceeded those for Pu(V+VI) by more than two orders of magnitude.

Based on comparisons with natural chemical analogues, the different oxidation states of plutonium are expected to show markedly different biogeochemical properties. Uranium and thorium, for example, are very similar in basic chemical properties to Pu(VI) and Pu(IV) respectively. Although the crustal abundances of the two elements are similar, uranium is present in open ocean seawater at a concentration 10^4 times higher than thorium[1]. Similarly, differences in direct biological accumulation are observed and, as Cherry and Shannon state[2], "thorium must, clearly, be taken up more efficiently by marine organisms than is uranium".

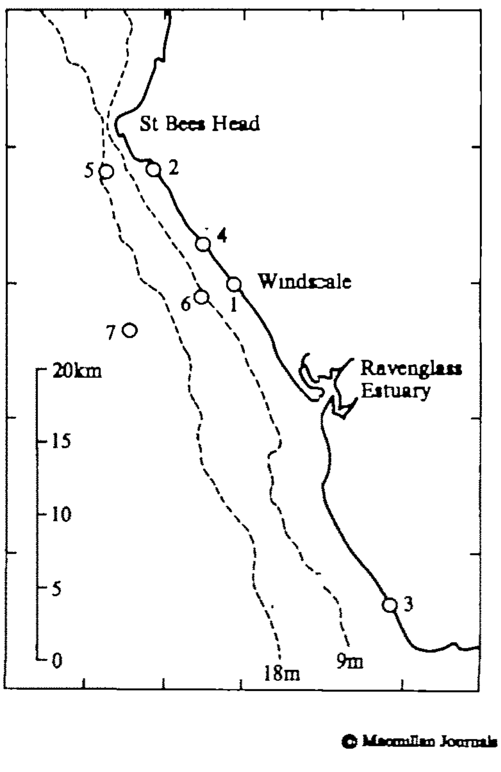
The potential importance of oxidation state in determining the environmental chemistry of plutonium has frequently been stated[3–6]. Analytical procedures to separate the oxidation states have been published[7–8] and applied to laboratory samples. Laboratory experiments have also used plutonium, in initially specified oxidation states, to test the stability[9], biological availability[10–13] and attachment to sediments[14] of various oxidation states. These observed biogeochemical differences between the chemical analogues of plutonium combined with the differences observed between actual plutonium oxidation states in laboratory experiments emphasise the importance of establishing the oxidation state(s) of plutonium found in the environment. However, we know of no information published regarding the actual oxidation states of plutonium found in natural seawater.

The BNF reprocessing plant at Windscale, Cumbria, discharges plutonium into the Irish Sea through a submerged pipeline terminating 2.4 km offshore. This release supports plutonium concentrations of about 1 pCi l^{-1} in filtered seawater near the discharge[15] and provides an excellent experimental area in which to study the oxidation state(s) of plutonium.

Eight seawater samples were collected from the seven locations marked on Fig. 1 during the summer of 1977. Shoreline samples were collected from water depths of ~0.5 m; offshore samples were collected either within 1 m of the surface or within 1 m of the seabed.

For each sample, the association of different oxidation states of plutonium with suspended particles was determined by measuring the concentration of each oxidation state in subsamples processed: (1) without filtration; (2) after filtration through a 0.22 μm Millipore membrane filter; and (3) in five of the eight samples after filtration through both 0.22 and 0.025 μm Millipore membrane filters. The method used for oxidation state differentiation was an adaption of the classical separation of Pu(III) and Pu(IV) from Pu(VI) by the co-precipitation of the former pair on a rare earth fluoride[16]. An internal measurement of the completeness of separation was provided by adding tracers of the relevant oxidation states to each sample and then monitoring the degree of separation of these tracers. Each subsample was made 0.8 M in HNO_3, 0.25 M in H_2SO_4, 0.5 mM in $K_2Cr_2O_7$ and 0.7 mM in $Nd(NO_3)_3$. Unfiltered subsamples stood overnight at this stage to allow exchangeable plutonium to leach from the particles before they too were filtered. Initial observations had shown that the leachate constituted >90% of the total plutonium originally on the particulate matter. Sufficient HF was added to raise the total fluoride concentration to 0.25 M and the resultant NdF_3 precipitate was removed by filtration. $(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$ was added to the filtrate

Fig. 1 Location of water sampling stations.



(5g l^{-1}) which then stood overnight to complete the reduction of any Pu(VI) in the sample. NdF_3 was again precipitated by the addition of 100 mg of Nd per litre and removed by filtration. The method was considered sufficient to disrupt any complex ions—organic or inorganic—initially present in the sample. The plutonium in each NdF_3 precipitate was purified by routine[17] techniques of anion exchange and individual plutonium isotopes were determined by α-spectrometry using silicon surface barrier detectors.

Completeness of the oxidation state separation and chemical yields were determined from the recovery of two isotopic diluents, ^{236}Pu(VI) and ^{242}Pu(IV), added to each subsample as soon as possible after collection (and after filtration for filtered subsamples). Each tracer was isotopically pure and contained >99% of its plutonium in the specified oxidation state. Overall chemical yields exceeded 80% and the separation of the two tracers typically exceeded 97%, even for the unfiltered subsamples. This maximum 3% deviation includes any plutonium which changes oxidation state during the analysis as well as any which simply carries on the incorrect precipitate.

Any Pu(III) or Pu(V) initially present would be rapidly oxidised by the $Cr_2O_7^{2-}$ to Pu(IV) and Pu(VI) respectively and would be indistinguishable from the original Pu(IV) and Pu(VI). The oxidation of Pu(IV) by dilute $Cr_2O_7^{2-}$ is an extremely slow process and was unimportant during these experiments. In a well oxygenated, high *p*H system, such as surface seawater, the higher oxidation state in both the Pu(III):Pu(IV) and Pu(V):Pu(VI) couples were assumed to be the more likely and subsequent results are reported as Pu(IV) and Pu(VI). A more detailed description and discussion of the method will be presented elsewhere.

The Pu(IV) and Pu(VI) concentrations, tabulated by particle size class in Table 1, were calculated for each sample using the plutonium concentrations measured in the subsamples. The concentration of plutonium on particles >0.22 μm was the numerical difference between the concentrations measured in the unfiltered subsample and the 0.22 μm filtered subsample. The concentration on particles between 0.025 and 0.22 μm was the difference between the concentrations measured in the two filtered subsamples. The concentration in water passing the 0.025 μm filter (0.22 μm in the initial three samples) is presented directly. These results indicate that plutonium retained on the filters is predominantly Pu(IV) and that plutonium passed in the filtrate is mainly Pu(VI). Simply filtering these samples provided a fair separation of the oxidation states, a feature which could prove useful in estimating the oxidation state present in previous measurements. Little of the plutonium of either oxidation state which passes a 0.22 μm filter is removed by the 0.025 μm filter, suggesting that the plutonium may be in true solution and not simply attached to very small particles. Furthermore, the ratio of Pu(VI) to Pu(IV) in solution, particularly for the offshore surface samples, is sufficiently constant to suggest first that the two are at or near oxidation–reduction equilibrium, or second, that the rate constants governing the approach to this equilibrium are slow compared with the initial rate of dispersion of Windscale effluent in the sea.

Assuming that there are heterogeneous exchange reactions for both Pu(IV) and Pu(VI) between seawater and suspended particulates, distribution coefficients (K_d = pCi kg^{-1} in particles/pCi l^{-1} in water) for each oxidation state as well as for total plutonium were calculated. These K_d values for each sample are given in Table 2. As the composition of suspended particulates is extremely variable, it is not surprising that the distribution coefficients are far from constant. However, the general trends are unmistakable. Although the distribution coefficients measured for total plutonium are typical of values previously reported for the Irish Sea[15] and other systems[18], in these samples the distribution coefficients for Pu(IV) exceed those of Pu(VI) by factors of up to 1,000. This is consistent with the behaviour of the chemical analogues, Th(IV) and U(VI).

Because of this large difference in the K_d value, the initial distribution of plutonium between soluble and particulate phases (including the seabed) in the sea should be highly dependent on the ratio of oxidation states in the source material. It has been observed at Windscale that approximately 95% of the plutonium discharged is rapidly removed to the seabed and that the remaining 5% is removed from the area by water transport in a manner indistinguishable from the transport of the soluble isotope ^{137}Cs (ref. 15). From the above data we suggest that this 5% is largely Pu(VI) and that its ability to remain in solution is not surprising. Measurements of oxidation state ratios in Windscale effluent as well as at various distances from Windscale (and by inference at various times after release) are in progress. Nevertheless, the results of the present study have shown: (1) that plutonium can exist in natural seawater (at least in the one area for which data are available) as a mixture of

Table 1 Pu(IV) and Pu(VI) concentrations in water and suspended particulates

Sample location and type	Particle size* (μm)	^{239}Pu(IV) (fCi l^{-1})§	^{239}Pu(VI) (fCi l^{-1})§	Pu(VI)/Pu(IV)
1 Shoreline†	>0.22	6,200±300	600±200	0.096±0.032
	<0.22	300± 40	1,250± 60	4.2±0.6
2 Shoreline†	>0.22	15,600±500	610± 90	0.039±0.006
	<0.22	70± 10	400± 30	5.7±0.9
3 Shoreline†	>0.22	8,400±600	280± 90	0 033±0.011
	<0.22	60± 10	220± 30	3.7±0.8
4 Shoreline‡	>0.22	15,900±900	200± 70	0.013±0.004
	0.025–0.22	8± 4	54± 28	—
	<0.025	66± 3	791± 20	12.0±0.6
5 Surface‡	>0.22	111± 8	7± 14	0.063±0.126
	0.025–0.22	1± 4	−8± 13	—
	<0.025	33± 3	242± 10	7.3±0.7
5 Bottom‡	>0.22	1,250± 52	28± 17	0.022±0.014
	0.025–0.22	−1± 6	−10± 8	—
	<0.025	83± 4	200± 6	2 4±0.1
6 Surface‡	>0.22	524± 19	−22± 18	−0.042±0.034
	0.025–0.22	78± 8	34± 19	—
	<0.025	49± 3	399± 13	8.1±0.6
7 Surface‡	>0.22	57± 7	22± 42	0.39±0.74
	0.025–0.22	5± 4	42± 18	—
	<0.025	54± 3	443± 13	8.2±0.5

* As defined by filtration through Millipore membrane filters; this gives only a general indication of particle size.

† Samples collected 21 June 1977; subsample size = 0.8 l.

‡ Samples collected 10 August 1977; subsample size = 4.0 l.

§ Total of ^{239}Pu + ^{240}Pu; ±1 s.d. counting error.

Table 2 Distribution coefficients of Pu(IV) and Pu(VI)

Sample	Saspended load (mg l^{-1})	K_d ^{239}Pu(IV)* ($l\,kg^{-1} \times 10^{-5}$)	K_d ^{239}Pu(VI)* ($l\,kg^{-1} \times 10^{-5}$)	K_d ^{239}Pu(total)* ($l\,kg^{-1} \times 10^{-5}$)
1	60	3.4±0.5	0.08±0.03	0.73±0.05
2	110	20±3	0.14±0.02	3.1±0.2
3	140	10±2	0.09±0.03	2.2±0.3
4	40	60±4	0.06±0.02	4.7±0.3
5 S	2	17±2	0.14±0.29	2.1±0.3
5 B	5	30±2	0.28±0.17	9.0±0.4
6	2	54±4	−0.28±0.23	5.6±0.3
7	2	5.2±0.1	0.25±0.47	0.8±0.4

* Total of ^{239}Pu + ^{240}Pu based on ±1 s.d. counting error.

oxidation states; and (2) that it distributes between the soluble and particulate phases in a manner dependent on this mixture. It is clear that a knowledge of the changes in oxidation state undergone by plutonium will be important in understanding the ultimate fate of this element in the environment.

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Catastrophic events in a surface mixed layer

THE formation and evolution of mixed layers in the surface waters of the oceans are important in many fields of study and much effort has been expended towards the development of predictive models of the wind-mixed surface layer[1,2]. These models parameterise small-scale high-frequency turbulent mixing processes, which are difficult to measure, in terms of more easily observed variables such as the wind stress. Because this parameterisation depends on hypotheses about the character of the turbulent mixing which dominates the transport of heat and momentum, it is vital to have a realistic concept of the turbulent processes in active mixing layers. It is often assumed that a major source of turbulence at the base of the mixed layer is the shear between the mean currents in the mixed layer and those in the thermocline. Simple shear turbulence of this sort often may be described by a flux-gradient relationship[3]. We have now found that although a flux-gradient description is adequate most of the time at the base of an actively mixing layer, during a storm isolated and relatively rare large-scale catastrophic mixing events, which cannot be described by a flux-gradient relation, can dominate transport. Although these catastrophic events occur infrequently, the vertical heat transport associated with them can dominate the continuous transport associated with flux-gradient processes.

While taking microstructure profiles at Ocean Station P (50°N, 145° W) during August 1977, we observed a storm of substantial intensity (maximum wind speed 20 m s^{-1}) which lasted 48 h. Temperature profiles were taken from the RV Oceanographer with the microstructure profiler (MSP)[4], a winged instrument which falls freely through the water measuring temperature, pressure, and conductivity. Sampling on 23 August began at 0415 GMT, about 24 h into the storm. During an 8-h sampling interval, 22 casts were made, each lasting about 5 min, with 10 to 60 min elapsing between casts. Since the vessel was by no means motionless during the storm, each cast must be considered as an observation of an independent realisation of the processes involved. No clear evidence of systematic mean temporal changes in the profiles can be seen during the sampling. Observation of mixed layer deepening during this period was obscured by 5–10 m amplitude internal waves.

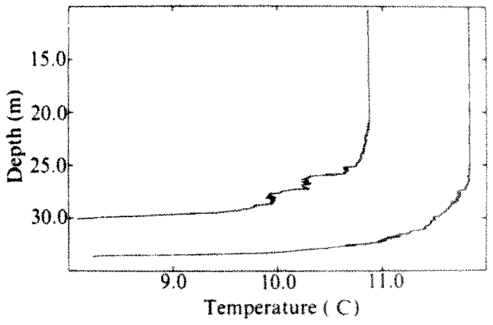

Fig. 1 Temperature profiles typical of small-scale, shear-generated turbulence at the mixed layer base. Left profile is offset 1 °C to the left. Thermocline depth difference is due to internal waves.

Most profiles on 23 August show the temperature to be nearly constant to within a few metres of the seasonal thermocline (Fig. 1). At the base of the mixing layer, a rather smooth transition from mixed layer to thermocline is usually seen; temperature structures seen here usually have a length scale less than several centimetres. This is characteristic of small-scale, shear-induced turbulence.

Two of the August 23 profiles are remarkably different (Fig. 2). They show events in which large masses of cool water are swept into the warmer isothermal layer above. In addition, two other profiles show large-scale disturbances at the mixed-layer base (Fig. 3). These may well be incipient events, in which the unstable mass of water has not yet separated from the water below.

Information regarding the vertical motion of these features can be obtained from the instantaneous fall rate of the probe. A winged vehicle with large drag coefficient responds rapidly to fluctuations in the local water velocity,[5] and drops at a constant speed with respect to the water in its immediate vicinity. (If the probe's fall rate is inferred from the pressure record, the measured speed of descent is insensitive to vertical velocities associated with surface waves.) On encountering the sharp

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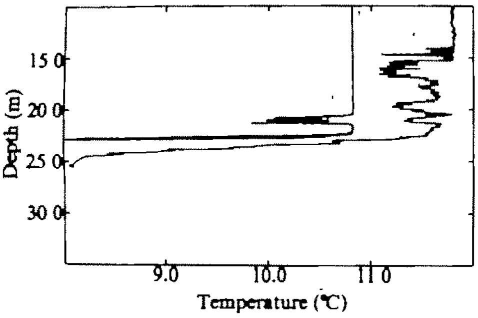

Fig. 2 Temperature profiles showing large-scale catastrophic mixing events. Event in left profile is very young, while event in right profile is older and in a state of collapse.

inversion in the profile on the left in Fig. 2, the probe abruptly ceased to fall relative to the surface, indicating that the water surrounding the probe was moving upwards at a speed comparable to the probe's vertical velocity, 12 cm s^{-1}. Thus, this water mass arrived from below only seconds before the observations, a conclusion borne out by the extremely large temperature gradients at its upper and lower edges. The event shown in the profile to the right in Fig. 2 was moving downwards at 5 cm s^{-1}, indicating that this water mass is older and in a state of collapse, consistent with the convoluted, somewhat diffuse character of its boundaries. The 'incipient' events in Fig. 3 each have an upward velocity of 2–3 cm s^{-1}.

The importance of the catastrophic events to mixed layer dynamics is revealed by a comparison with the more ordinary flux-gradient shear turbulence. An estimate of the heat flux at the base of the layer in the absence of large-scale mixing events is modelled as $H = -K_z \partial\bar{\theta}/\partial z$ where K_z is estimated from the Cox number[6] (the mean square temperature gradient normalised by the square of the mean gradient). In the absence of large events the heat flux at the base was of the order of 10^{-3} cal $\mathrm{cm}^{-2}\,\mathrm{s}^{-1}$.

The heat flux attributable to catastrophic events can be described by the free-turbulence estimate $H = K_e \Delta\theta/l$, where K_e is estimated from Richardson's law of diffusion as $1/2\varepsilon^{1/3}l^{4/3}$, ε is the rate of dissipation of mechanical energy per unit mass, l is the size of the disturbance, and $\Delta\theta$ is the temperature difference between the mass and its surroundings. The heat flux is then $H = 1/2\varepsilon^{1/3}l^{1/3}\Delta\theta$, weakly dependent on ε and l and strongly dependent on $\Delta\theta$. The dissipation rate may be estimated from Fourier analysis of the temperature gradient by finding the Batchelor scale, the smallest length scale at which temperature fluctuations are appreciable. Lower bound estimates of ε, l and $\Delta\theta$ lead to a lower bound heat flux for a 'typical' event of 0.3 cal $\mathrm{cm}^{-2}\,\mathrm{s}^{-1}$. Weighting the single-event heat flux by the probability of occurrence during the sampling period (0.1–0.2) yields a lower bound of 0.03 cal $\mathrm{cm}^{-2}\,\mathrm{s}^{-1}$ for an average heat flux. Thus the flux-gradient heat flux is dominated by the heat flux due to catastrophic events despite their rarity.

The energy available for changing the potential energy of the water column by deepening the mixed layer is the difference between the energy produced by turbulence and the energy dissipated by viscosity. Most of the energy produced by simple shear-stress is dissipated; thus this process is inefficient in accomplishing mixed layer deepening. Large-scale mixing events can, however, cause large rapid transfers of kinetic energy directly from the mean flow through interaction with internal waves[6], and thus be very efficient for mixed layer deepening. Instabilities in general are triggered when some dynamically important parameter, such as a Richardson number, reaches a threshold value. During a second milder storm on 31 August, apparently no threshold was reached, for no overturning events were found. We might therefore expect that realistic parameterisation of mixed layer dynamics in strong storms should include a threshold for the occurrence of catastrophic mixing events.

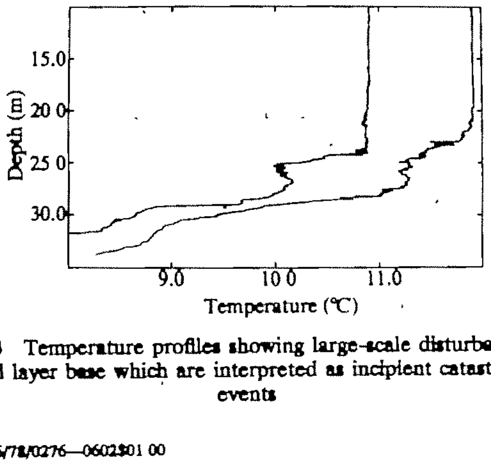

Fig. 3 Temperature profiles showing large-scale disturbance at mixed layer base which are interpreted as incipient catastrophic events

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^{230}Th/^{234}U age support of an interstadial sea level of −40 m at 30,000 yr BP

THE position of sea level about 30,000 years ago—in the Paudorf interstade in Europe[1] and the Plum Point interstade in northeastern America[2]—is disputed, estimates varying from above present sea level to ~50 m below it. The disagreement is due partly to ^{14}C dating problems, and partly to stratigraphic errors and incorrect tectonic assumptions[3]. We now confirm a previous estimate[4–6] of −40 to −42 m made from Huon Peninsula, New Guinea, and give new ^{230}Th/^{234}U data which support previous ^{14}C results.

Raised coral terraces at Huon Peninsula contain more late Quaternary reefs than similar formations elsewhere (such as in Barbados[7] and Timor[8]) because of higher uplift rates, and are the basis for the palaeo-sea level curve for the last 0.25 Myr given in ref. 9. We are concerned with Huon terrace II dated previously by ^{14}C at 28.5 ± 0.6 kyr (mean of two determinations[5] with seawater ^{14}C correction[10] subtracted). The ^{14}C dates are from massive clams, *Tridacna gigas*, which show very little inversion from aragonite to calcite and which are microscopically indistinguishable from *Tridacna* specimens known to be beyond the ^{14}C dating limit and which are measured as containing zero ^{14}C—hence the result of 28.5 kyr has been argued as being a satisfactory ^{14}C age estimate[5]. However, because of ^{14}C dating uncertainty for old shell samples reservations have been held about this result, in the absence of confirmatory ^{230}Th/^{234}U measurements[6].

A specimen of unaltered coral, *Favia speciosa*, collected from the crest of Huon reef II and Blucher Point (147° 40′E, 6° 10′S), 18 m above sea level, yields the following U-series results: U-content = 2.62 p.p.m., $^{234}\mathrm{U}/^{238}\mathrm{U} = 1.2 \pm 0.02$, $^{230}\mathrm{Th}/^{234}\mathrm{U} = 0.25 \pm 0.02$, age estimate = 31.0 ± 2.5 kyr. We believe that this reef crest is time-equivalent with that from which the ^{14}C dates were obtained (at 147° 37′E, 6° 7′S), based on continuous reef-tracing between the two sites. Analytic methods are explained in ref. 8.

We consider that these results confirm an age-estimate of 28.5 to 31.0 kyr for Huon reef II. In the context of rapidly rising Huon Peninsula, the associated palaeo-sea level must be estimated by subtracting (age × uplift rate) from present elevation of reef II. For this, Chappell[5] used a nonlinear uplift rate model; Bloom *et al.*[6] used a linear model. Reef II results differ little between these two models and indicate a paleo-sea level of −40 to −42 m. The structure of reef II indicates sea level rise before growth of the dated crest; a subsequent fall to about −140 to −150 m before post-glacial transgression has been interpreted from young submerged terraces and reefs[5]. A low sea level around −150 to −160 m, relative to this part of the globe about 17,000 years ago, is supported by dated submerged terraces off northern Australia[11,12].

These sea-level changes conflict with ice volume variations estimated from ^{18}O analysis of deep sea cores. Figure 1 compares Huon Peninsula sea levels for the past 45 kyr (refs 8, 9), with ^{18}O curves from Panama Basin core V19-28 (ref. 13) and Caribbean core P6304-9 (ref. 14) using the chronology of Shackleton and Opdyke[15] and Shackleton and Matthews[16]. Also shown are ^{14}C-dated variations of the southern margin of the Wisconsinan ice sheet[2]. Correlation of Huon Peninsula sea levels seems to be better with the ice margin changes than with the ^{18}O records. Note, however, that the ice margin curve is not zero-referenced, because the parameter discussed here is ice volume, which is very difficult to estimate before 17 kyr because of uncertainty about previous ice margin patterns[2,17].

Fig. 1 Sea levels relative to Huon Peninsula, δ^{18}O variations in Caribbean and central Pacific surface waters, and migration of southern margins of Laurentide ice sheet. Note that deep-sea core chronologies are based on interpolation[15] and may not strictly be comparable with the sea level and ice margin chronologies.

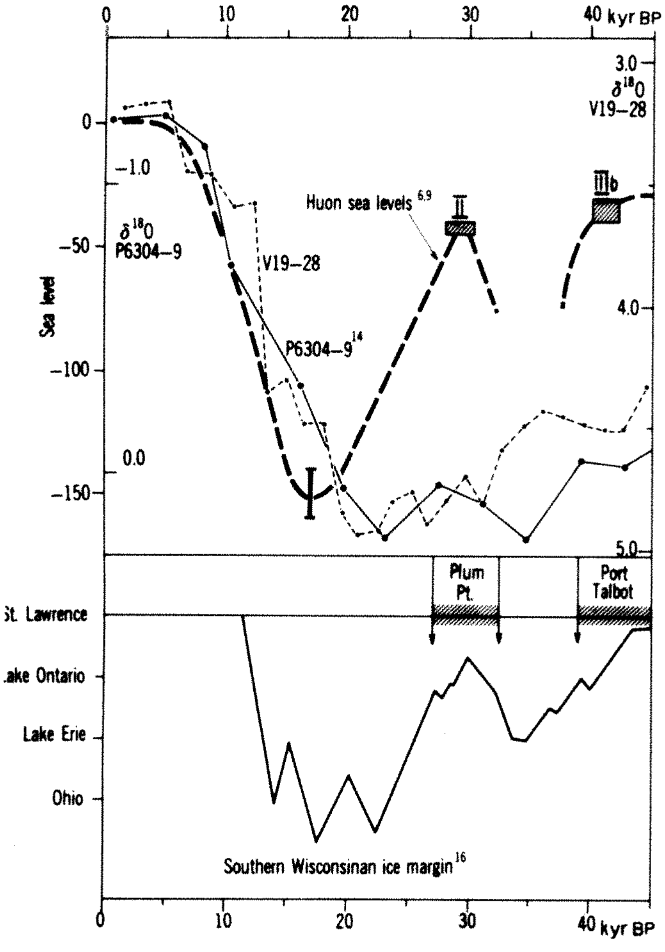

Table 1 Comparison of ^{230}Th/^{234}U and ^{14}C ages

Site	Ref.	C age (kyr)	No. of determinations	Th/U age (kyr)	No. of determinations
Huon P.	10	6.76	4	5.8	2
Huon P.	10	7.64	4	9.4	4
Gt Barrier Reef	11	13.7±0.2	2	17.0±0.8	2
Huon P.	This work	28.5±0.6	2	31.0±2.5	1
Huon P.	5, 6	35.6±1.5	2	40±3.0	4

Two factors damp the ^{18}O ice-volume signal in deep sea cores—seabed mixing processes, and variation of average ^{18}O content of the icecaps themselves[9]. However, the discrepancy for reef II, as well as for reef IIIb (ref. 5) (Fig. 1), is so large that one of the following three conditions must apply:

(1) Sea level relative to Papua New Guinea and northern Australia was no lower than about −75 m instead of the −150 m interpreted from dated submarine reefal terraces.

(2) A non-eustatic factor has affected the Huon Peninsula sea-level curve—either highly nonlinear tectonics, or hydro-isostatic processes greater than previously calculated[18,19], or some other process such as geoid changes[20].

(3) The δ^{18}O records are significantly smoothed by seabed mixing processes, reducing amplitude of peaks of less than ~10 kyr. Cores with higher sedimentation rates such as V19-28 show more variation than lower rate cores (compare V28-238)[15], and cores with yet higher rates may well offer a good test of our sea level curve.

Finally, we wish to comment on ^{14}C and ^{230}Th/^{234}U chronologies. Table 1 lists comparative data from several reefs—many from the same specimens—from ~6 to 45 kyr, ^{230}Th/^{234}U results beyond 6 kyr consistently show greater ages than ^{14}C. In the Holocene range the ^{14}C ages are more 'accurate' in terms of geochemical equivocations; however, they are well known to appear younger than true age due to past variation of atmospheric ^{14}C production. Concerning older examples in Table 1, corroboration seems to come from recent thermoluminescence dates from archeological materials ~30 kyr old, which like our reef II results, appear similar to ^{14}C dates from the same sites[21,22]. The problem of minor ^{14}C contamination in samples beyond ~30 kyr is difficult to eliminate, although we note that our ^{230}Th/^{234}U age for reef II is very close to the ^{14}C age of the Plum Point Interstade climax (Fig. 1), suggesting that the two chronologies become close at that time.

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Eastwards migration of the Tuscan anatectic magmatism due to anticlockwise rotation of the Apennines

AT the northern apex of the Tyrrhenian Sea, two mountain chains face each other displaying opposite vergence: the Corsican alpine chain and the Northern Apennines (Fig. 1). The Corsican alpine chain consists of both oceanic and continental nappes transported towards the West which are assumed to have derived from the peeling off of European, Tethyan and African (Austroalpine domains) lithosphere sectors. The age of metamorphism and orogenic transport towards the European foreland is mainly Cretaceous–Palaeogene. We present here an interpretation of the available data on post-collision continental anatectic magmatism in the Tuscan province (Italy) in the light of geodynamic evolution of the Tyrrhenian Sea.

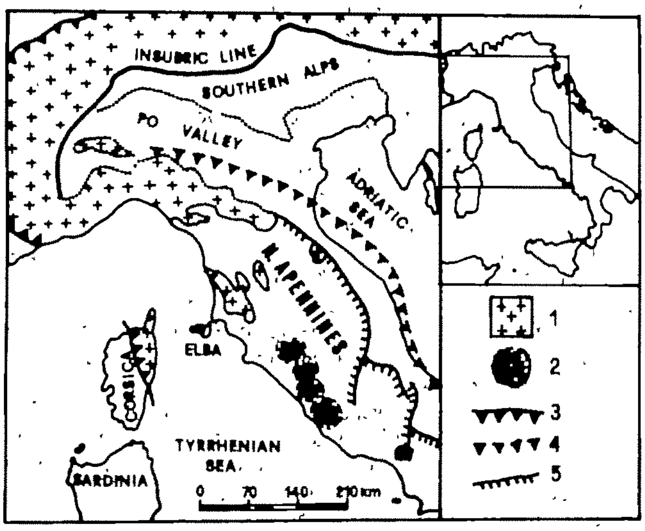

Fig. 1 Tectonic sketch map of northern Italy. 1, Palaeogene and Neogene Europe-verging Alpine nappes; 2, main upper Tertiary–Quaternary volcanoes; 3, European front of the Alpine chain; 4, front of the Apennines; 5, front of the carbonate units in the northern and central Apennines.

The northern Apennines consist of east and northeastwards transported continental nappes peeled off from the African (Insubric–Apulian domains) litosphere sector. Here, the age of metamorphism and orogenic transport is mainly Neogene. The Ligurian nappes, considered to represent slices of oceanic crust[1], are interpreted here as part of the Cretaceous–Palaeogene Europe-verging Alpine chain. During the late Oligocene and early Miocene they partially overthrust as a whole on to the Insubric domains that in Neogene times formed the Africa-verging Apennine chain (Fig. 2).

Extensive magmatic activity (the Tuscan province) developed between Corsica and Tuscany, within the strongly deformed belt marking the collision zone between the two chains. The crustal anatectic origin of this magmatic province is indicated both by petrological evidence (xenocrysts of quartz, cordierite and sometimes sillimanite, corundum normative composition[2] and isotopic data (high ^{87}Sr/^{86}Sr ratios[3,4] high δ^{18}O (ref. 5). Absolute age determinations range from 8.5 on Elba Island in the west to 0.43 Myr in Mt Amiata in the east[4,6] (Fig. 3).

The deep crustal structure in this area is controversial. The interpretation of gravimetric data[7] suggests crustal thicknesses under western Tuscany of $\sim$25 km. This value is too small for the root zone of a Neogene mountain chain, which implies that the crust here has been thinned and rifted after continental collision. A new interpretation has been proposed based on seismic results and the best fitting of seismic, gravimetric and magnetic data[8,9]: according to this model, the area between Elba and western Tuscany is characterised by the existence of two crust–mantle boundaries, the first 15–25 km deep and the second 45–50 km deep. The body between the two discontinuities might contain continental crustal material, a possible source for the observed magmatism. Accepting crustal thicknesses around 45–50 km the western Tuscany area might correspond to a normal or slightly thinned root zone for the northern Apennines.

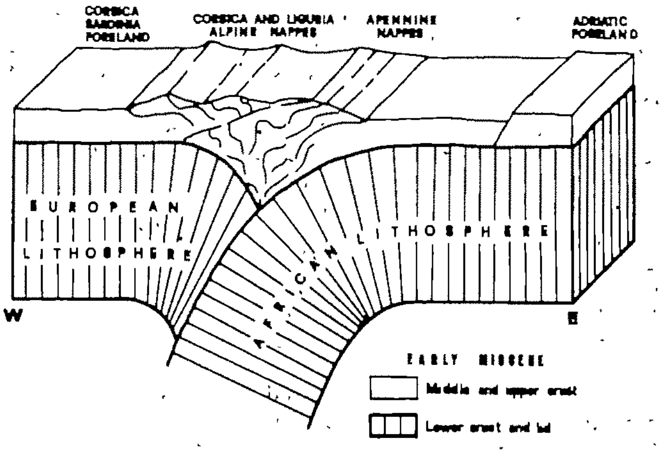

Fig. 2 Palinspastic resoration of the Corsica–Sardinia and Apulia margins at the end of Palaeogene.

Figure 3 shows all the published geochronological data available concerning the Tuscan province; new K/Ar age determinations on a peralkaline lamprophyre outcropping near Sisco (northeastern Corsica) are added here.

Velde[10] first defined the potassic peralkaline nature of the Sisco lamprophyres. The mineralogy is phlogopite, sanidine, olivine, quartz and an uncommon anphibole (potassic richterite). All the data strongly support the geochemical affinity of these rocks with those of the Tuscan province[11]. The dated samples were collected from the outcrop described and analysed by Velde[10] and belong to a sill intruded within the ophiolitic nappes (Schistes lustrés). Whole rock and separated phases were dated by the K/Ar method (Table 1). The errors (1σ) have been evaluated using the Cox and Dalrymple formula[12]. The K/Ar dates range from 14.3 to 15.4 Myr. In a plot of ^{40}Ar/^{36}Ar against ^{40}K/^{36}Ar they define an isochron of 14.2 ± 0.2 Myr with an initial ^{40}Ar/^{36}Ar value of 300.1 ± 0.6. This age is the oldest so far

Fig. 3 Simplified map of the Tertiary and Quaternary magmatic activity in the Tuscan province. Figures indicate the ages (Myr).

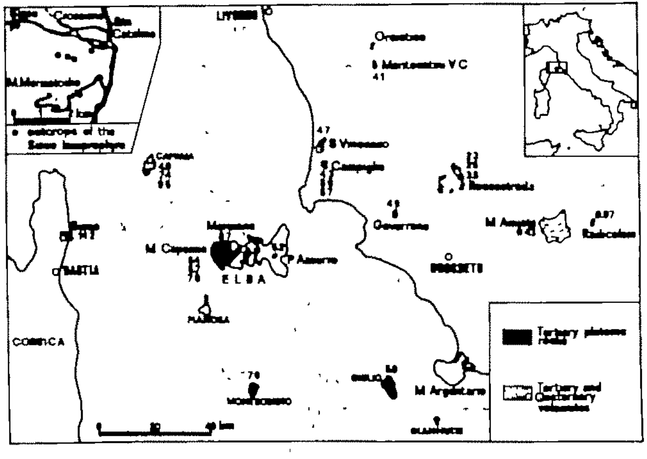

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Table 1 K–Ar ages of the Sisco peralkaline lamprophire*

Analysed phase	K (%)	^{40}Ar rad (%)	^{40}Ar rad ($\times 10^{-10}$ mol g^{-1})	^{40}Ar/^{36}Ar	^{40}K/^{36}Ar ($\times 10^3$)	Age (Myr)
Whole rock	7 15	57.6	1.82	696.3	481	14.3 (±0.4)
K-Feldspars	8.86	32.1	2.44	430.1	152	15 4 (±0.5)
Phologopite (60–100 mesh)	8.00	21.0	2.06	373.9	93.4	14.4 (±0.5)
	8.00	46.7	2.10	551.1	298	14 7 (±0.4)
Phlogopite (100–140 mesh)	8.11	21.1	2.23	372.9	87.3	15.3 (±0.5)
	8 11	27.9	2.13	409.0	132	14.7 (±0 5)

^{40}Ar was determined by isotope dilution mass spectrometry, using a GD-150 Varian Mat mass spectrometer and the ^{38}Ar-enriched Zurich spike. The calibration was checked by LP-6 USGS standard, a muscovite internal standard (MCT) and Bern LP-6 biotite. K was determined in duplicate by flame atomic absorption with a Perkin Elmer 303.

* $\lambda_\varepsilon = 0.585 \times 10^{-10}$ yr^{-1}; $\lambda_\beta = 4.72 \times 10^{-10}$ yr^{-1}; $^{40}K/K = 1.19 \times 10^{-4}$ atom/atom.

found in the north-Tyrrhenian post-Palaeogene magmatic province. Figure 4 shows these new data, and literature values, in an age against distance plot; the distances have been computed by projecting the magmatic centres on the Bastia–Radicofani line. The new data from North Corsica confirm the eastwards migration of the magmatism[4,6] giving 1.3 cm yr^{-1} as a mean migration velocity.

Contrasting models have been proposed to explain this age–space relationship. Alvarez[13] argued that the Tuscan magmatic activity belongs to a calc-alkaline sequence related to a former Benioff plane dipping eastwards under Italy. According to the Alvarez model the eastwards migration of the magmatism results from the subduction and simultaneous anticlockwise rotation of the Corsica–Sardinia microplate. Marinelli[11] postulated the existence of a hot spot under Tuscany ('Etruscan swell') and suggested that the eastwards migration of the magmatic activity is evidence of a movement of the Tuscan continental lithosphere towards the west with a velocity of about 1 cm yr^{-1}.

Our new model might explain better the genesis and the eastwards migration of the Tuscan magmatic activity, taking into account the main geodynamic events occurring in the Tyrrhenian area during Neogene and Pleistocene times.

The beginning of the Tuscan anatectic magmatism overlaps with the final phases of the Sardinian calc-alkaline volcanism[14]. This volcanism has been recently associated with the subduction of the continental Insubric–Apulian lithosphere dipping westwards beneath the Corsica–Sardinia block (Iberia plate)[15]. The Oligocene and Neogene Africa-verging nappes of the Apennines are interpreted, in this scheme, as peels detached from this subducing lithosphere and pushed toward the east and north-east[15] (Fig. 2).

Sialic material packed in the roots of the Apennine chain during the main compression phases might represent the source of the anatectic magmas. At that time, before the middle Miocene, the Tyrrhenian Sea had not yet developed as an extensional basin. Starting from middle–late Miocene times, tensional tectonics characterised the Tyrrhenian area, until an oceanic plain had developed by Messinian times[16,17]. The Tyrrhenian seafloor spreading is supposedly connected with an anticlockwise rotation of the Apennines[15]. According to this model, and assuming a rotation pole in the Tuscan area or further north, it is possible to reconcile the simultaneous continental magmatic activity in Tuscany with the tholeiitic activity on the Tyrrhenian abyssal plain during late Miocene and Pleistocene times.

The generation and ascent of the magma is probably a result of heat flow increase, pressure relief and fluid mobilisation during a period of tensional rift tectonics. The heat flow increase could be due to a hot spot[11] and/or a general rise of the isotherms in response to thinning of the lithosphere during rotation of the Italian peninsula. In any case, the considerable radioactive heat generation of the upper crustal material in the root zone should be taken into consideration. Furthermore isotopic and geochemical data reported for the Tuscan magmatic rocks support this model and suggest a mixing of upper crustal materials[2,18] with mantle derived magmas.

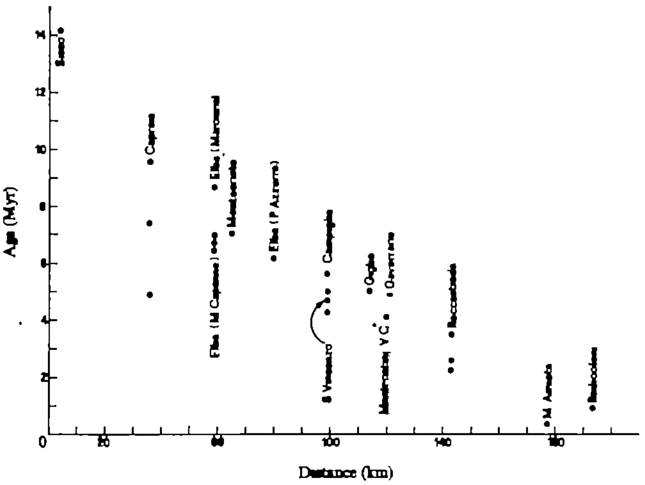

Fig. 4 Ages versus the distances of the magmatic episodes in the Tuscan province. The distances have been computed by projecting the magmatic centres on the Bastia–Radiocofani line.

The eastwards migration of the magmatism can be easily related to a progressive crustal thinning and subsequent rifting due to the anticlockwise rotation of the Apennines. The Tyrrhenian abyssal plain, being far from the rotation pole, is the area in which tensional tectonics are most apparent, so that thinning and rifting of the continental crust was followed by the generation of new oceanic crust in the south.

Finally, such a rotation model may explain the simultaneous late Miocene–Pliocene longitudinal rifting of the chain along the Tyrrhenian border and the nappe and fold development along the Adriatic front.

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Do aerosol anomalies precede earthquakes?

REPORTS of strange animal behaviour in my home village, which was struck by the Friuli earthquake in northeastern Italy (6 May 1976) convinced me that aerosol particles were electrostatically charged before the quake and that the physiological effects were essentially caused by an increase of the ion concentration in the air resulting in reactions such as the serotonin irritation syndrome. A mechanism is suggested here for the liberation of electrical charge from underground: electrochemical glow discharge.

The most remarkable animal behaviour effects (the quake occurred at 2100 LT) were the following (a more detailed description is given elsewhere[1]). Deer formed flocks—in the late afternoon, a flock of 15 deer came down from the mountains and close to the village, crowding together with apparently no interest in grazing—an event never before seen in this area. Cats left the houses and the village—at the time of the quake no cat was apparently left in the village. They did not return until two days later (there were many aftershocks). In three cases, cats dragged kittens outdoors and bedded them in green vegetation. Before fleeing from an apartment a cat was seen to move its ears convulsively for some time. Mice and rats left their hiding places—on one farm mice and rats were observed running around before the quake. People were annoyed and surprised as all their five cats were missing. Fowl refused to roost a few hours before the quake. People fleeing from their houses during the quake found their fowl already scattered in the garden. The animals had apparently forced their way out from the pen before the disaster. On one farm, several minutes before the quake, the fowl made such a noise that farmers thought a fox had entered the chicken house. Cattle panicked in their barns—according to many reports, cattle showed clear signs of fear 15–20 minutes before the quake. The animals started to bellow, tear at their chains and paw their boxes. Dogs barked without apparent reason. This behaviour started 20 min before the quake. Some people were guarding their property, suspecting intruders, just before the quake hit. Birds emitted calls at unusual times—one person heard a cuckoo, never normally heard at night. Caged birds started to fly—in six places people observed that 10–15 min before the earthquake birds became restless and occasionally emitted calls. Then they began to beat their wings and finally started to fly. A bird kept its plumage wet—in the late afternoon before the quake occurred, one woman observed that her canary had emptied the water bowl which was filled daily and generally only half emptied in one day, she refilled it and shortly before the earthquake found it empty again; the bird must have kept wetting its plumage.

The characteristic animal behaviour observed in northeastern Italy by people with little experience of earthquakes (independent accounts from neighbouring areas are published elsewhere[2]) is essentially identical to that reported from China[3–7]. This can be seen from a publication by the Earthquake Office in Tientsin[8]. It advises that an earthquake may be imminent when cattle, sheep or horses refuse to get into the corral, when rats run out from their hiding place, when chickens fly up to the trees and pigs break out from their pens, when ducks refuse to go to the water and dogs bark for no obvious reason, when snakes come out from the winter hibernation, when pigeons are frightened and will not return to their nests, when rabbits with their ears erect jump up and crash into things and when fish jump out of the water as if frightened.

There are many other accounts of anomalous animal behaviour before earthquakes (see refs 9–21): 33 independent reports from all over the world were collected by the US Department of the Interior[18] and I have collected 78 reports with abundant supporting evidence from folk tradition and mythology[1]. After the successful prediction of the earthquake of Haicheng in China in 1975 the dramatic increase in the number of reports of anomalous animal behaviour was quoted as one of the principal reasons, as well as foreshock activity and water level changes, for the declaration of the state of emergency.

In the search for scientifically useful clues to the phenomenon I studied the case of a livestock train standing in a small town (Pontebba) awaiting customs clearance on the evening of the earthquake. The wagons were boxes of sheet iron with several rectangular openings only. The cattle and horses had been fed and watered. Under normal circumstances they stay quiet. Approximately 15–20 minutes before the earthquake, however, the animals started to show discomfort and produce excessive noise. The transportation workers decided to notify their superiors, fearing poisoning or disease. These wagons (Faraday cages) cannot apparently shield these pre-earthquake signals. Nor could small vibrations preceding the quake have been responsible for the unrest. There was only one, relatively strong, foreshock within one minute of the main quake and how could animals that have been travelling by train for days suddenly get panic-stricken? Why are birds that live in the trees frightened and how can such hypothetical small vibrations be distinguished from similar ones produced by trucks and trains?

The clue to this mysterious animal behaviour was finally found, in my opinion, when I spoke to a retired precision mechanic. As the time of the earthquake approached he was repairing a small wrist-watch. One operation consisted in placing a thin lamella of stainless steel—estimated to weigh a few tenths of a gram—on the clockwork. It would not stay there but jumped from its place. After several futile attempts the confused man went and looked at the weather outside; the sky was completely cloudless. After resuming his work he still found the strong electrostatic repulsion phenomenon; immediately afterwards the first shock occurred.

Only charged aerosol particles could have caused such a strong charging of metal parts in the same polarity. It led me to hypothesise that before the earthquake an unknown geophysical phenomenon liberates electrostatic charges from underground.

The Menlo Park Conference on abnormal animal behaviour before earthquakes[18] gave this hypothesis some additional weight by advancing arguments against several alternative potential geophysical signals of non-electric origin. Underground noise would not be heard better by most domestic animals, as the conduction of ultrasound signals to which they are more sensitive is drastically attenuated during its passage through rocks. Changes of the magnetic field of the Earth as well as possible barometric pressure changes would be smaller than those variations which most animals would usually experience and microshocks would also be detected by seismographs apart from being too frequent to cause serious discomfort to animals.

This hypothesis is supported by much circumstantial evidence[1] among which the following is the most convincing.

(1) There are many reports of physiological effects of charged aerosol particles on animals and humans[22]. Much work has been done on the action of small air ions (as the smallest charged aerosol particles are usually called), especially by A. P. Krüger *et al.*[23,24], who succeeded in tracing the biochemical factor responsible for the action of air ions on living beings. Experiments have shown that small positive ions increase the production of serotonin, a powerful neurohormone, engaged in physiological functions such as sleep, moods and the transmission of nervous

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impulses. Subsequent investigations[25–28] supported these results and revealed that a typical kind of illness, felt during certain weather situations such as the Foehn in the Alps or the Sharav in the Near East, which are also characterised by abnormally high concentrations of positive ions, is also accompanied by a high serotonin production. The cluster of symptoms (migraine, nausea, vomiting, irritability, hyperperistaltis) was called the serotonin irritation syndrome. It can be successfully treated with negative ions or serotonin blocking agents.

(2) An electrostatic charging of aerosol particles can produce fog in air with less than 100% humidity[29]. Fogs or strange clouds have been explicitly designated as earthquake precursors since the days of Aristotle and Pliny, and there is a long record of such observations in Europe[10], America[1], and Japan[14] (chiki = the air from the ground). A detailed description of such a phenomenon is, for example, given by A. von Humboldt for an earthquake in Cumana, Venezuela[19].

(3) Light-producing electrical discharges are to be expected as a consequence of strong charging of aerosol particles. There are many reports of luminous phenomena during and before earthquakes.[9,14,19,20,30–33].

(4) The proposed electrical nature of the short-term earthquake precursory phenomenon makes it qualitatively comparable with transient electrical phenomena in the atmosphere preceding dramatic weather changes. Many characteristic features of reported animal behaviour are qualitatively similar before major storms[1]. It is possible that animals see in an earthquake a phantom storm. Their reactions might therefore partly be due to a weather instinct which is distorted by the unusual intensity in the development of the event and the physiological effect of air ions. This would explain the evolution of an 'earthquake sense' in animals in the absence of appropriate opportunities for natural selection.

(5) The atmospheric changes preceding strong earthquakes are apparently also felt by sensitive people. The term 'earthquake weather' is known in Europe, South America and in western US[1]. It is associated with an unusually warm and oppressive weather situation. The reported physiological reactions[9] are comparable to those felt during Foehn, Scirocco or Sharav, which are known to have been accompanied by high concentrations of positive air ions.

(6) There are old reports of qualitative measurements of the electricity of the air by means of electrometers during periods of aftershocks. One account is by A. von Humboldt[19] from Venezuela (1799) and one by V.-Eandi from Piemont[34,35] (1808). Both scientists report a marked increase of electricity during shocks. For both earthquake events there are parallel reports of anomalous animal behaviour. In certain descriptions of anomalous animal behaviour the electrostatic nature of the underlying phenomenon becomes directly evident. According to de Ballore[36], in Sicily it was said that an earthquake was imminent "when cats start crying, bristle their hair and sparks are released from their backs". Additional evidence is provided by the Chinese experience[6], that animals of very different temperament and sensitivity (58 species) are aware of approaching earthquakes. Remarkably, smaller animals (larger surface-to-volume ratio) are generally found to be more sensitive than bigger ones.

(7) The charged aerosol hypothesis does not only give an apparently consistent explanation of all important earthquake precursory phenomena from the popular tradition, which are occurring in the atmosphere, but is also consistent with an essential feature of the main earthquake theory of antiquity as elaborated by generations of Greek philosophers and especially by Aristotle. This claims that, before an earthquake, a strange gas (apparently warm and viscous) the 'pneuma' evolves from the Earth producing a series of atmospheric earthquake precursor phenomena (and the earthquake itself). The 'pneuma' of Greek philosophers could have been electricity of the air.

It is quantitatively justifiable to consider the piezoelectric effect in quartz-containing rocks (the average concentration of quartz in the Earth's surface is 15%) as the primary source of the electrostatic energy. There is reportedly sufficient alignment of piezoelectric microcrystals to produce macroscopic effects[37] and Finkelstein and Powell have estimated that many thousands of volts per metre could be generated by pressure waves of earthquakes (25–250 bar) (refs 38, 39). Attempts to explain earthquake lightning as a consequence of macroscopical electrical discharges through the air, however, failed to be convincing because of the measured low resistance of the ground (10^3 Ωm) compared with that of the air (10^9 Ωm). Electrical currents in the ground would rapidly neutralise separated charges.

These currents could, however, be the ultimate cause of charge liberation by inducing a phenomenon known as electrochemical glow discharge or glow discharge electrolysis[41,42]. The discharge is easily produced if the anode of an electrochemical cell is lifted slightly above the electrolyte's surface and the applied voltage simultaneously increased to maintain the electrical current across the gap. The resulting glow discharge can be maintained with a potential difference as low as 500 V and, if the gap is sufficiently thin even at atmospheric pressure (contact glow discharge[43]). Positive ions (mostly H_2O^+), accelerated towards the water surface will generate radicals and ions with yields comparable to that of α-particles. But attainable dose rates with mA-glow discharge currents can exceed that of conventional radiochemistry by a factor of 100–10,000 (ref. 42). Microscopic electrochemical glow discharges resulting from piezoelectric transient phenomena and the passage of electrical current across tiny fractures in rocks and thin water films also seem to provide a quantitatively satisfactory mechanism. Glow discharges in the range of 1 mA (telluric currents of this order of magnitude have been shown to occur in connection with earthquakes and volcanic eruptions[5,40]), with 3.7×10^{17} ions min^{-1} accelerated across 100 V towards a water surface could drastically change a typical ion concentration in the range of 10^4–10^6 cm^{-3} in the air above.

My consideration of many very old or inexact observations to support the proposed hypothesis does not meet present-day scientific standards and might provoke criticism. I believe this is unjustified as long as corresponding modern investigations do not exist. The question also arises as to whether our exclusive reliance on instrumental research (with the exception of China) and renunciation of a vast treasure of observations by scientifically unqualified people is justifiable in the case of earthquakes. In support of this view a remarkable fact should be mentioned: 1,900 yr ago the Roman naturalist and writer Pliny the Elder specified four different signals, which could indicate a threatening earthquake[44]. Three of these, the appearance of foreshocks, turbidity in water wells and frightened birds, are considered relevant today, as they were used to forecast the earthquake of Haicheng in China[3,5]. The fourth, the appearance of an atmospheric sign, a special kind of fog in an otherwise clear sky, could prove to be relevant if the hypothesis proposed in this paper were confirmed. Do we know more about short-term earthquake prediction than did the Romans?

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Evidence for domestication of the dog 12,000 years ago in the Natufian of Israel

THREE canid finds from the Natufian in the northern Israeli sites of Ein Mallaha (Eynan) and Hayonim terrace indicate a special man–animal relationship. These consist of a diminutive carnassial and mandible, and a wolf or dog puppy skeleton buried with a human. The finding of a puppy skeleton in such close association with man is of particular significance as an indication of a close relationship between man and dog.

A domestic animal is one whose breeding is largely controlled by man. Evolution of a domesticated species, therefore, results mainly from artificial selection, with natural selection playing a subsidiary part. The process of domestication implies the separating off of a breeding stock from its wild forebears. Most workers agree that the wild progenitor of the dog was the wolf *Canis lupus*[1–5].

A *Canis* skull from the Mesolithic site of Star Carr, England (9,538 ± 350 BP) was identified as a dog, on the basis of dental overlap[2]. Lawrence[3] identified dog from Idaho (USA, 10,400 BP) and from eastern Anatolia (~9,000 BP). More recently, Turnbull and Reed[6] suggested that a mandible from the Zarzian (~12,000 BP) of Palegawra cave (Zagros mountains, Iraq) belonged to a dog, on the basis of dental overlap and small size.

The finds we report here come from the Natufian, an Epipalaeolithic culture (formerly referred to as a Mesolithic culture) and dates to 12,000–10,000 yr ago. The Natufians, whose culture was limited to the Levant, are considered to have been hunter-gatherers, and were the first peoples to live in circular dwellings in what were perhaps the earliest permanently settled villages. Their sites, compared with those of preceding periods are larger, occupying areas of 1,000 to 3,000 m^2, and contain numerous pounding stones (pestles and mortars) as well as, for the first time, large cemeteries. Art forms are also found, as is a highly sophisticated tool kit, consisting largely of microliths, including lunates. They were the antecedants of the first agriculturalists in the region.

Bate's claim[7] for the domestic status of a *Canis* skull from the Natufianof El Wad cave (Mount Carmel, Israel) could not be confirmed[8]. Recent excavations at two Natufian sites in northern Israel, Mallaha (Eynan) and Hayonim terrace, provide what we believe to be more concrete evidence for some kind of special relationship, perhaps domestication, between man and dog/wolf in this early period, around 12,000 BP.

The site of Mallaha, near the old Huleh lake in the upper Jordan valley, has been under excavation since 1955 (ref. 9). Two phases of Natufian occupation have been distinguished[10]: the canid osteological remains, a mandible from a living floor and a puppy from a tomb, both come from the lower one. This has so far revealed three superposed half-buried dwellings, originally probably circular and 9–11 m in diameter, whose northern halves are missing. The walls, some preserved to a height of more than 1 m, were built of limestone blocks, piled more or less vertically. The flint industry is characterised by lunates rarely less than 1.5 cm in length, most of which bear 'Helwan retouch', indicating an Early Natufian assemblage. Basalt pestles and mortars are fairly frequent, as are bone points, needles, pendants and some bone sickle handles.

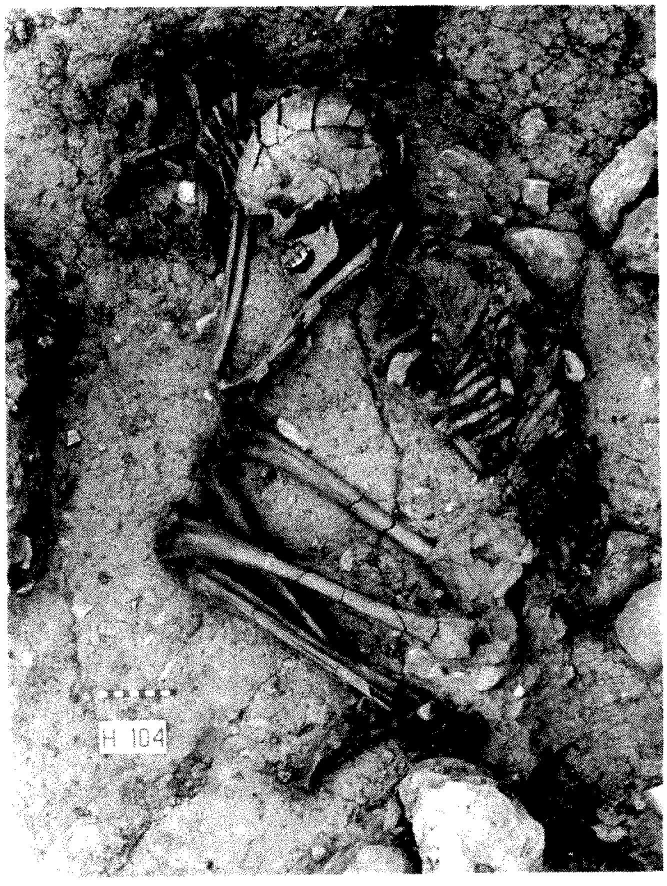

Fig. 1 Tomb H. 104 at Mallaha, showing the human skeleton and puppy.

In the entrance to dwelling 131, the oldest so far excavated in Mallaha, a large slab of limestone was found on the floor. This has been interpreted to indicate the presence of a burial (H. 104, ref. 11) especially due to its proximity to a human skeleton which lay 25 cm below it. The human skeleton, whose sex cannot be determined because of the damage of the pelvis, lay flexed on its right side, and judging by the state of its dentition, was an old individual. Its left wrist was partially under its forehead, and hand upon the thorax of a puppy, which has evidently been buried complete with the human (Fig. 1). All the milk teeth of the puppy are fully erupted, and of the long bones exposed, a humerus has both epiphyses open, indicating that the age at

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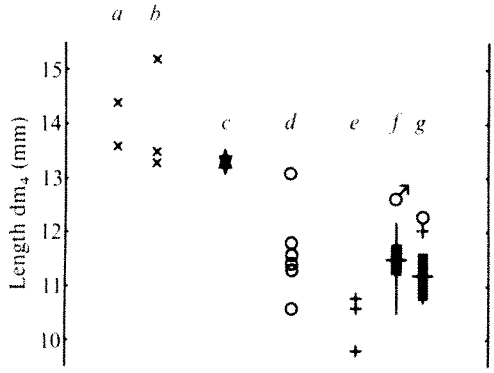
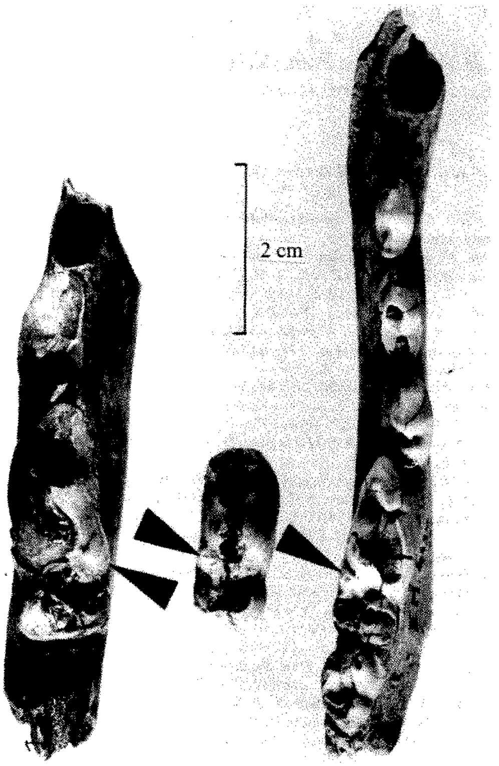


Fig. 2 Maximum length (mm) of the last lower milk molar (dm$_4$, milk carnassial) of canid puppies. *a*, Two wolves from Turkey; *b*, three wolves from Israel; *c*, the Mallaha puppy; *d*, six dogs from Turkey, Israel and Egypt; *e*, three jackals from Turkey; *f*, 14 male jackals from Israel, range, mean ±95% confidence limits; *g*, six female jackals from Israel, range, mean ±95% confidence limits. (Data from Turkey supplied by S. Payne).

death was between 3–5 months[12], while the advanced state of wear of the lower milk carnassial makes an age of 4–5 months more likely. The upper teeth are too damaged to be measured, but the dm$_4$ which is intact, has a maximum crown length of 13.3 mm. Comparison with the same tooth of recent Israeli and Turkish wolves, jackals and dogs (Fig. 2) indicates that the fossil puppy was either a dog or a wolf, but not a jackal, being outside the size range of the latter.

An adult canid mandible was found in dwelling 131. It came from an undisturbed living floor, characterised by seven post holes, and lay not far from two hearths containing bones which included a human cranium and a pair of gazelle horn cores. The mandible is complete from canine to second molar and belongs to a large canid. The metaconid on the M$_1$ is poorly developed (Fig. 3) as in wolf and dog; unlike the jackal metaconid which protrudes lingually and is more prominent[1,13].

Fig. 3 Occlusal views of the Hayonim terrace carnassial (centre) and Mallaha mandible (right) compared with an Upper Pleistocene wolf mandible fragment (P$_4$ and M$_1$) from Ein Gev IV (Jordan valley, Geometric Kebaran A period). The M$_1$ metaconids are arrowed.



Osteological separation of the dog and wolf is problematical, but tooth crowding and smaller size have been used as criteria for the identification of dogs. It has frequently been claimed that dog jaws are slightly shorter than those of their wolf progenitors, resulting in a certain degree of dental overlap, particularly of the lower fourth premolar and the lower first molar[2,3,6]. This dental overlap was analysed using an index of overlap (crown length of M$_1$ ÷ alveolar length from P$_4$ to M$_1$). The value for the Mallaha mandible is 0.67, indicating some degree of overlap (Fig. 3). A collection of nine recent dogs from Israel and Egypt, however, gave values from 0.65 to 0.70 (mean 0.68) and a collection of 34 recent wolves from Israel gave values from 0.62 to 0.67 (mean 0.65), with six specimens actually possessing values of 0.67. Another index, the length M$_1$ ÷ alveolar length of the lower premolars, showed a similar overlap between dogs and wolves. These indicate that dental overlap criteria are not very efficacious in dog–wolf separation, and are probably to some extent dependent.

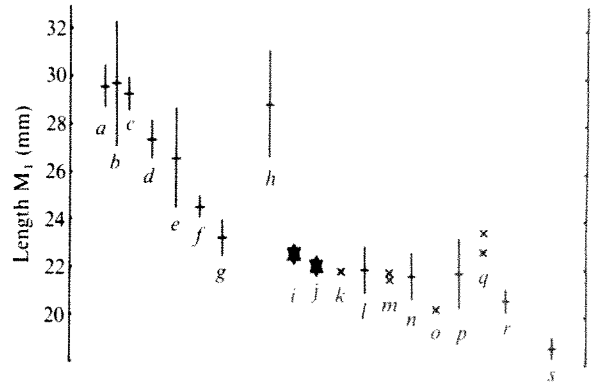


Fig. 4 Antero-posterior length of M$_1$ of recent and fossil canids. These show the north–south size cline in recent palaearctic wolves (*a* to *g*); the Pleistocene–Holocene diminution in the Levant (*h* versus *f*) and the wolf–dog differential (*f* versus *i* to *r*). Samples are represented by their mean ±95% confidence limits, single specimens by a star or cross. Recent wolves: *a*, Greenland and Ellesmere land, $n = 12$ (ref. 20); *b*, Russia and north Scandinavia, $n = 4$; *c*, Denmark, Holocene and recent, $n = 7$ (ref. 20); *d*, Turkey, $n = 9$; *e*, Zagros mountains, $n = 5$ (ref. 6); *f*, Israel, $n = 26$; *g*, Arabian peninsula, $n = 7$. Fossil wolves and dogs *h*, Ksar Akil U. Pleistocene, $n = 4$ (ref.21); Tabun B, $n = 1$ (ref. 7); and Ein Gev IV, $n = 1$ combined; *i*, Mallaha mandible M$_1$; *j*, Hayonim terrace M$_1$; *k*, Palegawra *Canis* (ref. 6); *l*, Jericho Pre-Pottery Neolithic to Early and Mid Bronze Ages, $n = 7$ (Ref. 17); *m*, Cayonu, east Anatolia, 9,000 BP (ref. 3); *n*, Korucutepe, east Anatolia, Bronze Age, $n = 12$ (ref. 22); *o*, Arad, north Negev, Early Bronze Age; *p*, Persian period of Israel, $n = 6$; *q*, two male dogs from Suez, Egypt; *r*, dogs from archaeological sites in Hungary, $n = 42$ (ref. 23). Recent jackals: *s*, Israel $n = 24$.

The terrace of Hayonim, in the western Galilee, was excavated by O. Bar Yosef in 1966 and 1969, and by D. Henry in 1974–75 (ref. 14). Over a thin layer assigned typologically to the Geometric Kebaran A, lies a Natufian assemblage. From the lowermost level of this a single ^{14}C date of 11,920 BP (SMU 231) is available. The canid tooth reported here is a lower carnassial from the trial trench (square D depth 30–50 cm, 1974 season's excavation) and is located within the Natufian. Its crown is chipped at the anterior end, and like the M$_1$ of the Mallaha mandible described above, is morphologically wolf-dog, with a poorly developed metaconid (Fig. 3).

The sizes of the M$_1$'s of both these finds are compared with other wolves and dogs both geographically with recent wolves from the Palaearctic region, and temporally with Near Eastern Upper Pleistocene to recent wolves and dogs (Fig. 4 and Table 1). The results of this analysis indicate firstly that recent wolves increase in size with increasing latitude in accordance with Bergmann's rule, and secondly that Upper Pleistocene wolves from the Levant were larger than recent ones, so that the wolf, like the mole rat, gazelle, goat, cat, fox, hyaena, wild boar and

Table 1 Recent and fossil canids, maximum length in millimetres of the lower carnassial, M_1

		N	Mean	s.d.
Recent wolves:				
Greenland and Ellesmere land[20]		12	29.6	1.46
Russia and north Scandinavia		4	29.7	1.67
Denmark, Holocene and recent[20]		7	29.3	0.76
Turkey		9	27.4	1.05
Zagros mountains, Iraq[6]		5	26.6	1.7
Israel males and females		26	24.6	1.07
Israel males		25	25.0	1.54
Israel females		13	24.3	0.94
Arabian peninsula		7	23.2	0.73
Fossil wolves and dogs:				
Ksar 'Akil[21]	Upper Pleistocene	4	28.8	2.66
Tabun B[7]	Mousterian 40,000–45,000 BP	1	29.5	
Ein Gev IV	Geometric Kebaran 13,000–14,000 BP	1	28.6	
Ein Mallaha	Early Natufian c. 12,000 BP	1	22.6	
Hayonim terrace	Early Natufian	1	~22.2	
Palegawra[6]	Zarzian 12,000 BP	1	21.9	
Jericho[17]	Pre-Pottery-Neolithic-Bronze Age 10,000–4,000 BP	7	22.0	1.09
Cayonu East Anatolia[3]	9,000 BP	2	(21.7 & 21.8)	
Korucutepe East Anatolia[22]	Bronze Age	12	21.7	1.53
Arad Israel, North Negev	Early Bronze Age 4,800 BP	1	20.3	
Israel	Persian period 2,500 BP	6	21.8	1.38
Male dogs, Suez, Egypt	Recent	2	(23.5 & 22.7)	
Archaeological sites in Hungary[23]	Neolithic–17th Century AD	42	20.7	1.62
Jackals, recent:				
Israel, males and females		24	18.7	0.88
Israel males		20	18.6	0.82
Israel females		12	18.6	0.89

aurochs decreased in size at about the end of the Pleistocene[15–18]. Dogs from later archaeological sites in the Near East are even smaller than recent wolves. Thus three size classes of the wolf–dog line exist in Israel—Upper Pleistocene wolves, recent wolves and domestic dogs. The problem, therefore, is to which of the latter two size classes the Mallaha and Hayonim finds belong. Combining the two M_1 measurements, and comparing them with modern Israeli wolves, the probability that they belong to the latter is <0.01 (Student's *t* test) and we conclude that on this basis they are dog rather than small wolves.

Having distinguished three size classes for the Israeli Upper Pleistocene–Holocene wolf-dog line, we postulate two independent factors as having been responsible for each of the two size declines: (1) the temperature elevation at the end of the Pleistocene, which we associate with the difference between Upper Pleistocene and recent wolves, and (2) domestication resulting in the difference between recent wolves and dogs.

These finds are important in that they support evidence from other arachaeological sites for interaction between man and canids in the Epipalaeolithic. The puppy, unique among Natufian burials, offers proof that an affectionate rather than gastronomic relationship existed between it and the buried person, an addition to our knowledge of the way of life of Natufian hunter-gatherers. The contemporary diminutive adult carnassials, of size equivalent to dog and not wolf, indicate that *Canis lupus* had, by the early Natufian, been subject to some degree of selective breeding by man. It would seem unlikely that taming alone would be sufficient to bring about this kind of change in the animals' dentition. We suggest, therefore, that domestication of the wolf occurred 12,000 years ago, before that of the food animals such as goat, sheep, cattle and pig, which were probably not domesticated until one or more millenia later[19]. If this hypothesis is correct, then together with Starr Carr in England, and Palegawra in Iraq, we might conclude that it was 'man-the-hunter' who first domesticated the wolf, another social carnivore.

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Note added in proof: The following dates pertaining to the puppy burial level at Mallah have been received from the University of Lyons radiocarbon laboratory: Ly 1660 11,590±540 BP; Ly 1661 11,740±570 BP; Ly 1662 11,310±880 BP.

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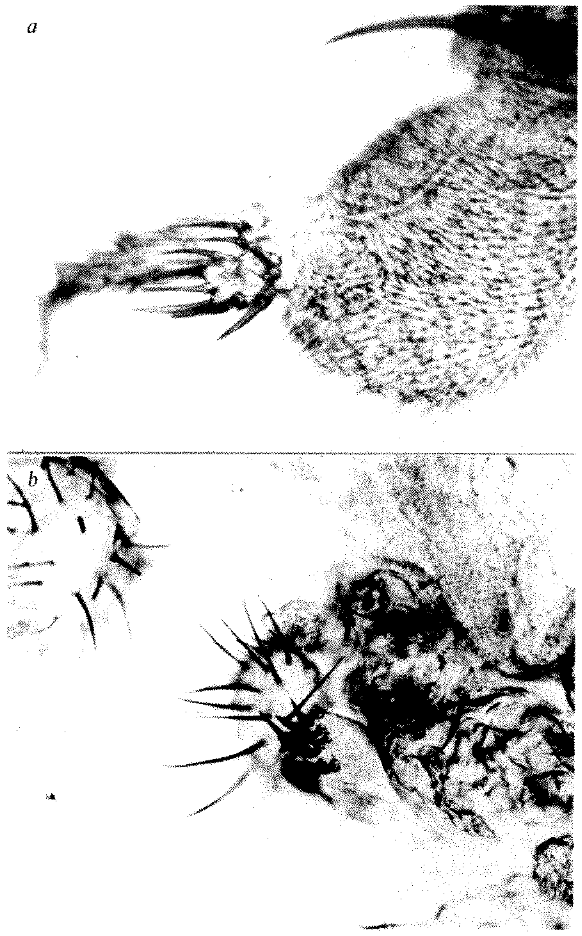
Tumorous head is a maternal-effect homoeotic mutant of *Drosophila melanogaster*

THE homoeotic mutants of *Drosophila* are of special interest in developmental biology because they cause the substitution of one body segment by another in the adult. The antennae of *tumorous head* (*tuh*) flies are replaced by mesothoracic leg tissue and part of the head by genital structures[1,2] (Fig. 1). Homoeotic mutants are thought to be the result of mutations in regulatory genes[3] which cause a suppression of the activity of one set of genes and the activation of an alternative set to bring about such a specific, well-defined transformation. These mutations somehow cause a group of cells at some stage in the life cycle to change their developmental pathway and differentiate during metamorphosis into different structures from those which they would normally form. We have analysed the *tumorous head* mutant and report here that it is probably the consequence of a gene acting at the time of determination.

Homoeotic genes act at one of several possible stages in development to bring about the observed phenotypic changes[3]. They could alter the initial determination of the cells, so that early in development, around blastoderm formation, when cells are becoming committed to follow specific pathways in development they become leg and genitalia instead of antenna and head. Many homoeotic genes need to be active throughout development[4,5], so that when determined cells divide the daughter cells also have the same developmental commitment. Thus, homoeotic genes could be envisaged which alter the maintenance of determination, in this case the cells would initially be determined correctly but because of the mutant gene products are unstable and change determination during development. Other genes could act at the time of differentiation by incorrect interpretation of the state of determination. *Bithorax* (*bx*) which transforms the haltere to wing is needed throughout development and probably affects the maintenance of determination[4,5]. *Contrabithorax* (*Cbx*)[6] which transforms the wing to haltere acts late in development and is also probably affecting the stability of cell determination.

Fig. 1 *a*, Third antennal segment transformed to leg; *b*, rostral-haut transformed to genitalia.



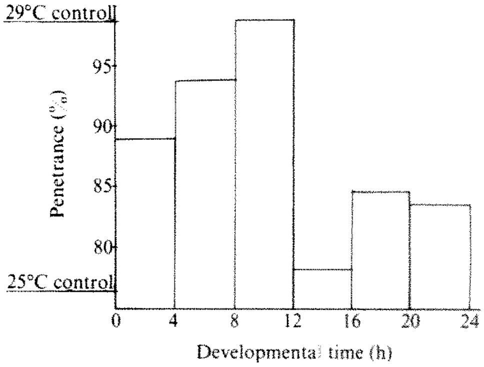


Fig. 2 Temperature sensitive period during embryogenesis of *tuh-3*. To establish a t.s.p. embryos laid by *tuh-3* mothers were shifted to 29 °C for 4-h pulses. Embryogenesis in *Drosophila melanogaster* takes 24 h to complete, thus the following pulses were applied: 0–4, 4–8, 8–12, 12–16, 16–20 and 20–24 h after oviposition. Temperature shifts later in development indicated that there is no temperature sensitivity for post-embryonic stages. Between 100 and 400 adults were examined for each pulse applied and a similar response was obtained in 3 independent repeats of the experiment.

The first few hours of embryogenesis in *Drosophila* are under the control of components laid in the egg by the mother [7–9] and hence depend directly on maternal gene activity. Cells develop according to their location in the embryo[10,11]. One could therefore expect that a gene affecting the initial determination of the cells would act during early embryogenesis or during oogenesis. A mutant in this class may well be a maternal effect mutation.

Table 1 *Tumorous head* is a maternal-effect homoeotic mutant

Female parent	Male parent	Number of flies scored	Penetrance (%)
tuh-3/*tuh-3*	wild type	323	18
wild type	*tuh-3*/*tuh-3*	465	0
tuh-3/*tuh-3*	*tuh-3*/*tuh-3*	714	53

Our experiments with *tumorous head* suggest that it is an unusual homoeotic mutation which fulfils the requirements of a mutant having its effect at the time of determination. There has been considerable genetic analysis of *tumorous head*, especially concerning the role of modifiers, enhancers and chromosome rearrangements[12,13]. We took the original *tuh-1a*; *tuh-3* stock which contained some flies with tumorous head phenotype under the control of a third chromosome mutation (*tuh-3*) and a modifier on the first chromosome (*tuh-1a*). We isolated *tuh-3* from this stock and established a line free of known enhancers. Our homozygous *tuh-3* stock had a penetrance (percentage of flies showing tumorous head phenotype) of 70% in contrast to those of previous workers[3] where the penetrance was 3%.

The *tuh-3* stock behaved as a maternal-effect homoeotic mutant. When homozygous *tuh-3* females were crossed to wild-type males some of the progeny showed the tumorous head phenotype but the reciprocal cross of *tuh-3* males to wild-type females gives no tumorous head phenotype. The gene also seemed to be active in early embryogenesis as *tuh-3* females

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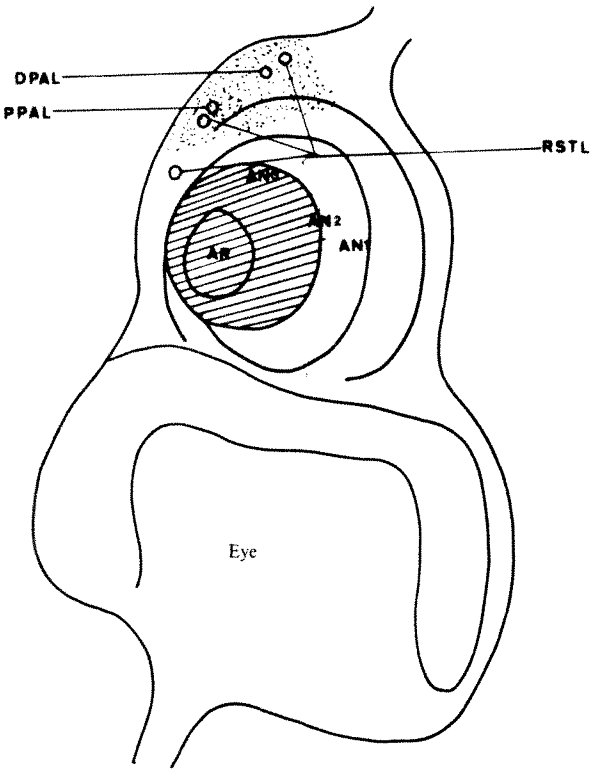

Fig. 3 Eye-antennal fate map indicating regions which give rise to the genitalia and leg transformations. Various regions of the disk were cut and implanted into host larvae where they metamorphosed with their host. Implants were scored for homoeotic transformations. In 33% of implants recovered from fragments containing the arista and third antennal segment we found leg tissue present and in 33% of implants recovered from fragments containing the rostralhaut region we found genitalia tissue present. RSTL, rostralhaut; AN1, AN2, AN3, 1st, 2nd and 3rd antennal segments respectively; DPAL, distal palpus bristles; AR, arista; PPAL, proximal palpus bristles. Stippled area, region that transforms to genitalia; hatched area, region that transforms to leg.

crossed to *tuh-3* males give more tumorous head progeny than when they are crossed to wild-type males (Table 1).

The temperative sensitive period (t.s.p.) of mutants is thought to be indicative of the time at which the gene products are being activated, thus the gene must be active before t.s.p. (refs 14,15). *Tumorous head* is temperature sensitive during embryogenesis and the pulse giving the highest *tumorous head* penetrance is 8–12 h after oviposition (Fig. 2). This then would agree with the *tuh-3*$^+$ gene being involved in determination.

Interestingly *tumorous head* transforms cells in the eye-antenna imaginal disk into cells from two different disks located in different body segments[16], the second leg disk in the mesothoracic segment and the genital disk in the last abdominal segment. All other known viable homoeotics act by altering cells from one disk to one other segment only[15,17] (for example, *bithorax* transforms metathoracic disk cells into mesothoracic disk cells[4]) or to another compartment of the same structure (for example, *engrailed* transforms posterior wing cells into anterior wing cells[18]). The mutant *proboscipedia* transforms the proboscis to antenna or leg, but not simultaneously to both structures in one fly; and two lethal mutants have been described which transform from genitalia to leg and antenna[19].

We have cut up the eye-antenna disks of *tumorous head* larvae (one can detect those with outgrowths in late third instar disks) and forced them through metamorphosis in a larval host[20]. In this way we have mapped the area of the disk involved in the homoeotic transformation (Fig. 3). The two regions involved are in extremely close proximity in the eye-antenna disk, although not necessarily in the embryo[21], but they undergo homoeotic transformations to very different segments of the fly.

As *tumorous head* is maternally controlled and has its t.s.p. early in embryogenesis, we would like to suggest that it is a candidate for a homoeotic gene acting at the level of embryonic determination.

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Genetic variability and population structure of *Poecilia latipinna*

THE pattern of genetic variability among natural populations is commonly attributed to the influence of environmental heterogeneity on natural selection[1,2]. However, I report here that in *Poecilia latipinna* (Pisces: Poeciliidae), a small ovoviviparous fish, genetic variability is more closely related to local population structure, in particular, the adult sex ratio.

P. latipinna inhabits the brackish water lowlands along the Atlantic and Gulf coasts of the US and Mexico. Fourteen population samples were collected by hand seine in the northernmost two-thirds of the species range between South Carolina and Texas. For each population, the numbers of each sex were counted, and phenotypic males were assigned to one of four classes based on the development of primary and secondary sexual characters. These classes were: (1) immature male—anal fin only partially transformed into a gonopodium (male copulatory organ); (2) mature male—gonopodium completely formed, but no secondary sexual characters acquired; (3) transformed male—mature individuals possessing one or more secondary sexual characters: elongation of second ray of pelvic fins, and anterior displacement of pelvic girdle; (4) dominant male—fully developed complement of secondary sexual characters, including a large, sail-like dorsal fin (fin rays at least long enough to reach the base of the caudal fin). Both field and laboratory observations have shown that the sailfin character has a significant role as an indicator of social status during sexual and agonistic encounters[3,4].

The presumptive genotypes of all individuals in each population were determined by starch-gel electrophoresis of 21 cellular enzymes and two nonspecific muscle proteins. Seventeen allozyme loci were polymorphic in at least one population (frequency of most common allele was 0.99). On average, 43%

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of the loci were polymorphic in each population. Within populations, the genetic variability (expressed as $\bar{H}$, the mean proportion of heterozygous loci per individual) was 3.6–10%, which is comparable to similar measures in other fishes[5].

The extensive geographical range of *P. latipinna*, encompassing a diversity of temperate to tropical climates, offers an excellent opportunity to investigate the influence of environmental heterogeneity on genetic variability. However, the pattern of population-level genetic variability does not seem to be a simple function of any discernible environmental gradient. The regression of $\bar{H}$ against 13 climatic variables (water temperature—mean high, low and range; water density—mean high, low and range; rainfall; pan evaporation; heating degree days, 18 °C baseline; solar radiation; length of breeding season—water temperature >20 °C (ref. 6); number of days with temperature above 32 °C or below 0 °C)[7,8], both singly and in combination, did not show any statistically significant relationships. On the contrary, inspection of the geographical distribution of genetic variability (Fig. 1) shows that low levels of $\bar{H}$ can occur in both equable (KW) and rigorous (MB) extremes of the species range. Similarly, both highly seasonal (MB, MC, RV) and highly stable (KW) environments display reduced genetic variability. The San Marcos population (SM), which shows the highest level of genetic variability in the species ($\bar{H} = 10.0$), occupies a remarkably stable hot-springs habitat (annual temperature range 22–22.5 °C)[9,10]. In light of such contradictory results, one might reasonably conclude that genetic variability in *P. latipinna* is responding to some unrecognised factor which is not an obvious component of local environmental heterogeneity.

The measure which successfully reflects interpopulation changes in $\bar{H}$ is the sex ratio of breeding individuals, which in this study is calculated as the number of females per dominant, or sailfin, male (Fig. 2; Kendall's rank correlation = −0.65, $0.001 < P < 0.01$). In *P. latipinna* populations, the number of dominant males, both past and present, can be reliably determined because the sailfin character is a permanent morphological change. In other poeciliid species which form male dominance hierarchies, the accessory display signal possessed by high ranking males is usually some form of distinctive coloration[11–19], which may diminish, or even disappear, with the loss of social status[13–19]. The number of dominant males in a population is of interest because many investigators have proposed that social dominance reinforces male reproductive success, even to the extent that dominant males may nearly monopolise the insemination of females[13–19]. If this concept of dominance and its role in the regulation of mating activity is applied to *P. latipinna*, then the explanation for the relationship between sex ratio and $\bar{H}$ is straightforward: as the proportion of dominant males in a population decreases, the paternal genetic contribution becomes channelled through fewer individuals. Thus, for any constant population size (N), as the sex ratio of breeding individuals becomes more skewed, the local effective population size (N_e) declines[20], and consequently genetic variability is reduced[21–23]. Previously, population size reduction[24,25] and reproductive behaviour[26–28] have been used to explain low levels of genetic variability in other organisms, but in *P. latipinna* the ratio of females to dominant males combines both of these concepts in a single direct measure of population structure.

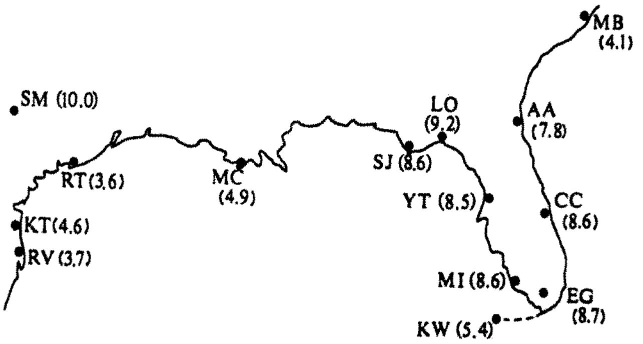

Fig. 1 The geographical location and the value of mean heterozygosity, $\bar{H}$, for 14 populations of *P. latipinna* from the Atlantic and Gulf coasts. Collection sites and sample sizes are as follows: South Carolina: Myrtle Beach (MB, 46); Florida: Jacksonville (AA, 53), Cape Canaveral (CC, 63), Key West (KW, 96), Everglades (EG, 25), Marco Island (MI, 50), Yankeetown (YT, 64), Live Oak Island (LO, 81), Port St Joe (SJ, 16); Louisiana: Morgan City (MC, 36); Texas: San Marcos (SM, 128), Rockport (RT, 61), Kingsville (KT, 73), Brownsville (RV, 256). Electrophoresis and enzyme assays performed on crude tissue extracts produced the following enzymes: eye esterase, lactate dehydrogenase (LDH); liver esterase, LDH, isocitrate dehydrogenase (IDH), phospho-gluco-isomerase (PGI), indophenol oxidase (IPO), peptidase (glycyl-tyrosine); muscle esterase, LDH, malate dehydrogenase (MDH), IDH, PGI, phospho-glucomutase (PGM), α-glycerophosphate dehydrogenase, creatine kinase, aldolase, peptidase (glycylglycine), nonspecific muscle protein. See ref. 4 for details of techniques.

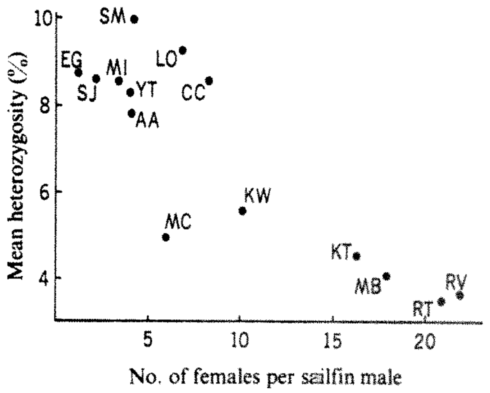

Fig. 2 Mean heterozygosity, $\bar{H}$, is inversely proportional to the ratio of females to dominant (sailfin) males.

It is possible that the observed sex ratios are not reflections of the true population structure, but are merely the artefacts of sampling technique or differential habitat selection by the sexes (or social classes). In this study such effects are minimal because the shallow streams and ponds where *P. latipinna* is found are usually small enough to obtain thorough samples. Even if one accepts the observed sex ratio as an accurate representation of local population structure, it could still be argued that the range of genetic variability in these samples is primarily determined by differences in gross population size. Without a careful census or identification of reproductive units this proposition is difficult to contest. However, rough estimates of gross population size at each sample locality can be obtained by taking the product of population density and habitat area. Such calculations show that to a first approximation the expectations for the effect of gross population size on $\bar{H}$ are not fulfilled: the two largest populations sampled in this study (KW and RV) fall at the low end of the range of heterozygosity; likewise, the two smallest populations (EG and SJ) are among the most genetically diverse. Reproductive substructuring within large, continuous populations could account for some instances of low genetic variability[29,30], but it is improbable that these objectively smaller populations (EG and SJ) are part of a larger (but undetected) panmictic unit. Another alternative would be that $\bar{H}$ is a function of the number of individuals in each sample, but no correlation was found between these two factors ($r = 0.19$; $P > 0.50$).

The appearance of a close association between population structure and genetic variability suggests that the interactions which regulate the local abundance of dominant males may be important adaptive features of poeciliid population biology. However, sources of variation in population structure need not be limited to intraspecific mechanisms. For example, studies of other poeciliids have shown that predatory fishes can significantly alter male demography by selectively feeding on the larger, showier males[11,16,31]. As no large, potential predators were found to be sympatric with *P. latipinna* in these collections, the observed sex ratios are probably not greatly affected by this type of interaction.

In *P. latipinna* mean heterozygosity varies not with the degree of environmental heterogeneity, but with the proportion of dominant males in a population. This relationship is most easily explained in terms of differences in local effective population size brought about by the hierarchical regulation of male mating activity.

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Arginine vasopressin maintains ethanol tolerance

THE neurohypophyseal hormone, [Arg⁸]vasopressin (AVP), like some of its analogues and smaller peptides of related structure, has the capacity to influence memory-related processes through direct action in the central nervous system[1–4]. These peptides have relatively long-lasting effects on memory consolidation or on retrieval of information[1,5]. It has been suggested that development of functional tolerance to drug effects involves an adaptive mechanism in the CNS similar to that which occurs during learning and/or memory consolidation[6,7]. We therefore wished to extend our studies[4] of the central actions of the neurohypophyseal hormones to include an investigation of the influence of these peptides on various aspects of functional tolerance to ethanol. One of the most consistently demonstrable effects of the neurohypophyseal peptides with respect to memory is the ability to inhibit the extinction of a conditioned avoidance response[1–3,5]. We now present evidence that treatment of animals with AVP during and after chronic ethanol administration leads to long-term maintenance of tolerance to the hypothermic and sedative effects of ethanol.

Male C57BL/6 mice were fed a liquid diet containing 7% (v/v) ethanol or an equicaloric amount of sucrose (controls) for 7 days[8]. On the morning of the eighth day, all mice were offered the sucrose-containing liquid diet (withdrawal). Using this regimen, we have previously shown that tolerance to the hypothermic and sedative effects of ethanol and signs of physical dependence were evident after withdrawal of ethanol from the animals' diet[8]. In the present experiments, animals received daily subcutaneous (s.c.) injections of 10 μg of AVP or oxytocin in 0.1 ml saline, or saline alone, throughout the drinking period and for 9 days after withdrawal. Animals were not injected with peptides on the day on which the ethanol-containing diets were removed, since the animals undergoing withdrawal were subject to handling-induced seizures. Four groups of animals were included in each experiment. The first group consumed the sucrose-containing diet and received saline injections. The second group consumed the sucrose-containing diet and received hormone injections. The animals consuming the ethanol-containing diet were similarly divided into a group receiving saline injections and a group receiving hormone injections. During the drinking period, ethanol consumption, intoxication and blood alcohol levels[8,9] were monitored daily. After removal of the ethanol-containing diet, withdrawal hypothermia and behavioural hyperexcitability[8] were observed at 2-h intervals for 12 h and blood alcohol levels were determined at 2-h intervals for 6 h in certain animals[9]. Tolerance to ethanol, determined by the hypothermic response and duration of loss of righting reflex after an intraperitoneal (i.p.) injection of 3 g per kg ethanol[7] was evaluated 24 h after withdrawal, and at 3-day intervals (starting on the third day after withdrawal) for 18 days thereafter. During tolerance testing ethanol metabolism was monitored in randomly selected mice from each group.

Blood alcohol levels during the drinking period and the clearance of alcohol after withdrawal and during tolerance testing were similar in all animals receiving the ethanol-containing diet. Signs of physical dependence, evident in the mice after ethanol withdrawal, also seemed to be similar in animals given saline, AVP or oxytocin[10].

Functional tolerance was first determined at a time when overt withdrawal signs had subsided (24 h after withdrawal). Figure 1 shows the time course for disappearance of tolerance after animals were withdrawn from the ethanol-containing diet. At 24 h and 3 days after withdrawal, the response to the challenge dose (3 g per kg) of ethanol was significantly less, both in terms of hypothermia (Fig. 1*a*) and 'sleep time' (Fig. 1*b*), in all groups of animals which had consumed the ethanol-containing diets as compared to their respective controls. The response of control animals which had received AVP treatment was not significantly different from that of control animals which had received saline at any time tested (Fig. 1); the same was true for control animals which had received oxytocin (data not shown).

At 6 days after withdrawal of ethanol, animals injected with saline were no longer tolerant to the sedative or hypothermic actions of ethanol (Fig. 1 and see ref. 8); that is, their responses were not significantly different from controls. Ethanol-consuming animals which had been treated with oxytocin also were no longer tolerant. In contrast, at 6 days after ethanol withdrawal, animals which had received AVP remained significantly tolerant to the effects of ethanol, as measured by both change in body temperature and sleep time in response to the challenge ethanol dose. This tolerance persisted during the time that the animals were treated with AVP (Fig. 1), and when AVP treatment was discontinued, at 9 days after withdrawal, ethanol tolerance disappeared with a time course similar to that seen in the saline and oxytocin treatment groups. Thus, at 3 days after the end of AVP treatment (12 days after ethanol withdrawal), animals still showed a significantly decreased response to the hypothermic and sedative effects of ethanol, but by 6 days after the end of AVP treatment (15 days after withdrawal), sleep time was not significantly different from that of controls, and the hypothermic

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response was returning towards control values. At 18 days after withdrawal, no differences in response were seen between control and ethanol-treated animals injected with AVP (Fig. 1*a*, *b*).

Thus, while the half-life for disappearance of ethanol tolerance after chronic ethanol treatment was approximately 3 days in untreated[8] or saline-treated mice, treatment with AVP maintained this tolerance for as long as hormone administration was continued. Since AVP treatment did not affect the rate of ethanol metabolism either during the drinking period, during withdrawal, or during tolerance testing[10], it is apparently functional (CNS) tolerance to ethanol which is affected by the neurohypophyseal peptide. Treatment of the animals with an equimolar dose of oxytocin, a peptide which differs structurally from vasopressin by only two amino acids (Fig. 2), had no such effect on ethanol tolerance. These results are in agreement with studies on the effects of the peptides in inhibiting extinction of an active avoidance response[1], and with our previous studies regarding the ability of neurohypophyseal hormones to attenuate puromycin-induced amnesia in Swiss-Webster mice[4,11]. In our studies, vasopressin attenuated the amnesia at a dose level at which oxytocin was ineffective[11]. One has to consider that peripherally administered peptides which differ in their primary structure may be metabolised by different pathways[12] and may not be distributed in an equivalent manner within the CNS. On the other hand, oxytocin has been shown to modulate the development of functional tolerance to morphine in rats when administered s.c. at doses which were even lower than those used in our studies[13].

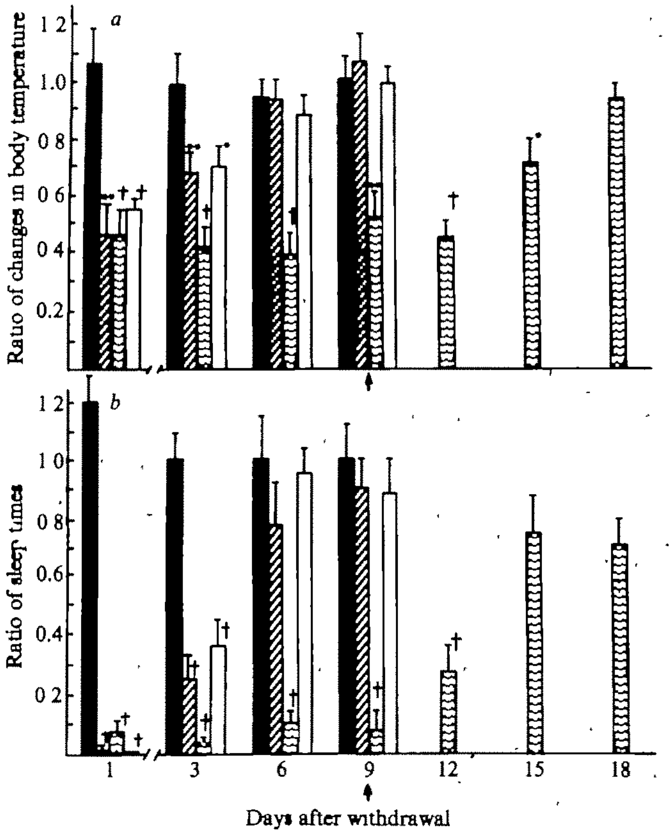

Fig. 1 Response of male C57BL/6 mice to a challenge dose of 3 g per kg ethanol, measured as decrease in body temperature (*a*) or duration of loss of righting reflex ('sleep time'; *b*). Data are presented as the ratio of the response of the ethanol-fed animals which had received various treatments to the response of their respective controls. The ratio of responses of control animals treated with AVP to those treated with saline is also shown. These ratios are less than 1.0 when the ethanol-withdrawn animals are tolerant to the effects of ethanol. As tolerance disappears the ratio approaches 1.0. Ratios were computed by dividing individual responses of animals in the group represented in the numerator by the mean response of animals in the group represented in the denominator; the value in the figure represents the mean ± s.e.m. of the ratios generated. ■, Controls: AVP-treated/saline treated. ■, Ethanol-dependent, saline-treated/saline-treated controls. ▨, Ethanol-dependent AVP-treated/AVP-treated controls. □, Ethanol-dependent oxytocin-treated/oxytocin-treated controls. Significance was determined by comparison (*t* test) of each ratio with the ratio of AVP- to saline-treated controls. Ratios of ethanol-dependent AVP-treated to AVP-treated controls on days 12, 15 and 18 after withdrawal were compared to the AVP/saline control ratio on day 9 after withdrawal, the day that AVP administration was discontinued (↑). †, $P \leq 0.001$; **, $P < 0.01$; *, $P < 0.05$. There were 6–12 animals in each group. The duration of the loss of righting reflex for saline-treated control animals when tested on day 1 after withdrawal was 25.1±2.1 min and body temperature was depressed by 2.2±0.2°C by the challenge dose of ethanol. These values for the AVP-treated control group were 25.8±3.1 min and 2.1±0.2 °C and for the oxytocin-treated controls, 23.4±2.8 min and 2.1±0.3 °C. Similar values were obtained with animals of these groups at other times during the period of testing.

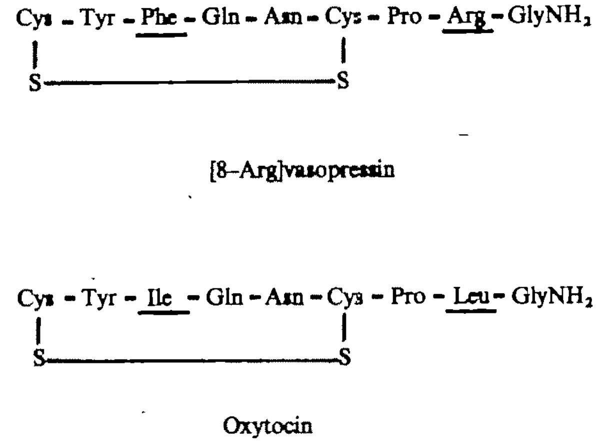
Cys - Tyr - Phe - Gln - Asn - Cys - Pro - Arg - GlyNH₂
S
S
[8-Arg]vasopressin
Cys - Tyr - Ile - Gln - Asn - Cys - Pro - Leu - GlyNH₂
S
S
Oxytocin

Fig. 2 Primary structure of arginine vasopressin and oxytocin. Nomenclature of peptides is in accordance with recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature[22]. AVP (430 IU mg^{-1}, rat pressor assay[23]) and oxytocin (506 IU mg^{-1}, avian vaso-depressor assay[24]) were supplied by Dr C. W. Smith (Department of Physiology and Biophysics, University of Illinois).

The evidence that neurohypophyseal peptides affect memory-related functions by direct interaction with elements of the CNS has been previously reviewed[1]. We have also found[14] that after s.c. injection of a dose of ^{14}C-labelled AVP[15] comparable to that used in the present study, detectable amounts of radioactivity were present in brain as intact hormone; such results are consistent with an action of AVP in the CNS. Although the doses of AVP administered to our mice were high compared with circulating levels of this hormone, the rapid peripheral metabolism of the peptide hormones dictates the use of doses significantly higher than intraventricularly administered doses which are capable of producing similar behavioural effects. Direct interactions between ethanol and AVP could have contributed to the observed effect of the hormone on ethanol tolerance. During the drinking period, both AVP and ethanol were present in the animal simultaneously. However, further studies in our laboratory[16] have indicated that when AVP injections are begun on the day after ethanol withdrawal, tolerance is still maintained for as long as the AVP treatment continues.

Acute exposure to ethanol inhibits the release of antidiuretic hormone[17], and a recent study showed a rebound increase in urinary AVP excretion after clearance of a single dose of ethanol[18]. It seems that tolerance to the effect of ethanol on AVP secretion did not develop after chronic ethanol treatment[18]. Plasma AVP levels have been reported to be increased in rats at the end of a regimen of repeated ethanol treatments[18] and in chronic alcoholic patients withdrawing from ethanol over a period of 5 days[19]. Furthermore, a selectively bred strain of alcohol-preferring rats, which was demonstrated to have both higher innate tolerance and higher acquired tolerance to ethanol than outbred rats or a strain of alcohol-avoiding rats[20], has also been found to excrete more AVP during 6 h after ethanol intubation than the alcohol-avoiding rats[21]. Thus, the ability of AVP to maintain tolerance to the sedative and hypothermic effects of ethanol after chronic ethanol treatment, which was observed in the present study, may reflect a physiological role of the hormone in modulation of ethanol tolerance.

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Increased spontaneous activity following substance P infusion into A10 dopaminergic area

THE polypeptide, substance P, has a discrete regional distribution in mammalian CNS; particularly high concentrations are found in the mesencephalon, close to the origin of the two major ascending dopaminergic pathways[1,2]. Two further observations which may reflect functional neurotransmission are, first, that substance P is released from spinal cord and hypothalamus[3,4], and second, that it has been localised using fragmentation techniques in nerve ending particles of bovine hypothalamus and substantia nigra[5,6]. Further support for this hypothesis comes from iontophoretic studies in which application of substance P into the medial nucleus of amygdala and substantia nigra induced a strong neuronal activation[7,8]. It has also been shown biochemically that the activity of dopaminergic systems may be modulated by substance P neurones[9,10]. The ventral tegmental area (VTA), which contains the cell bodies of the dopaminergic A10 neurones (DA-A10), receives a substance P innervation mainly from substance P cell bodies localised in the medial habenular nucleus[11,12]. DA-A10 neurones give rise to the mesolimbic DA system and also to the mesocortical DA axons innervating cerebral cortical area[13,14]. We have studied the behavioural effects of local application of substance P into the VTA, to try to elucidate the physiological role of substance P innervation of this area. A strong enhancement of spontaneous activity was observed following bilateral infusion of substance P. A major feature of this enhancement was increased exploratory behaviour. Moreover *d*-amphetamine-induced behavioural arousal was potentiated by previous infusion (1 h) of substance P into the VTA.

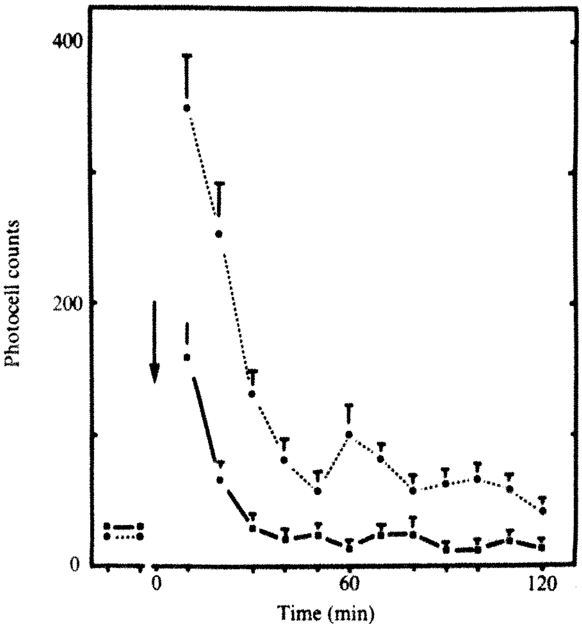

Fig. 1 Effect of acute bilateral infusion into the ventral tegmental area of isotonic saline (——) or substance P (– – – –) on whole body locomotor activity measured in photocell cages. The ordinate indicates the mean photocell counts + s.e.m., the abscissa indicates time by 10-min periods. Arrow, time of infusion. The lines before the arrow reflect baseline activity of the rats during 20 min before injection. The photocell cages were 70 cm × 25 cm and had two beams, which, when broken, registered counts in a recorder outside the testing room. Substance P was infused as a concentration of 3 μg in 1 μl ($n = 14$) and 1 μl of saline was infused ($n = 12$).

Forty-three Sprague–Dawley rats (300–350 g) were implanted with bilateral stainless steel guide cannulae (23 gauge) aimed 3 mm above the VTA. Coordinates were (in mm): 3.5 posterior to bregma, 0.5 lateral from midline, and 5.6 below skull surface (incisor bar 5 mm above interaural line). Histological examination revealed that the 86 infusion sites were well localised in the VTA. Ten days after surgery, 3 μg synthetic substance P or the solvent (1 μl of 0.9% sterile saline) were injected bilaterally by lowering an injection needle (30 gauge) through the guide cannulae to a point 8.6 mm below skull surface; the infusion lasted 120 s and a 60-s diffusion time was allowed before removal of the needles. The substance P dose used in our experiments is considerably higher than the endogenous amounts of this substance in the VTA; however Jessel *et al.* have shown that there is no reuptake mechanism at substance P terminals and that substance P is rapidly catabolised by peptidases in the extracellular space[4]. The experiments were carried out during the least active part of the rat's circadian rhythm (1200–1700), with white noise (67–68 db), constant temperature (20 °C) and illumination (0.14 ft lamberts). Rats were familiarised to the experimental conditions before testing began.

In the first experiment, photocell cages were used to measure whole body locomotor activity. On a test day, the rats were habituated to the cages for 1 h; then saline or substance P infusion into the VTA was made and the rats were returned

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immediately to the photocell cages for 2 h. Fourteen rats received synthetic substance P (Bachem) and 12 received saline. Animals injected with substance P in VTA exhibited a pronounced long-lasting increase in spontaneous activity (Fig. 1); analysis of variance indicated a significant group effect ($F = 28.4$, d.f. = 1,24, $P < 0.0001$) and group × time interaction ($F = 35$, d.f. = 11,11, $P < 0.001$). As soon as 20 s after the beginning of a substance P infusion, rats developed a behavioural excitation which was characterised by rearing, sniffing and hypermotility. The strongest effect was apparent during the first 40 min, but it was still in evidence during the subsequent hour.

In order to analyse this behavioural activation further, a second experiment was performed in a white square open field. Since rats have a tendency to avoid the centre area of the open field because of increased 'emotionality' or fear, we measured locomotion and rearing along the walls as well as in the centre area during a 10-min period. Eleven subjects were used as their own controls and were tested on four consecutive days. On the first, third and fourth test days, rats received bilateral infusion of saline into the VTA; substance P was injected on the second test day and all categories of behaviours analysed were enhanced after application (Fig. 2). Analysis of variance over 10 min revealed a significant activation of the following behaviours: locomotion periphery ($F = 5.4$, d.f. = 3,30, $P < 0.01$), rearing periphery ($F = 5.4$, d.f. = 3,30, $P < 0.01$), locomotion centre ($F = 13.9$, d.f. = 3,30, $P < 0.01$), and rearing centre ($F = 6.5$, d.f. = 3,30, $P < 0.01$). Interestingly, motor activity was not homogeneous throughout the open field, and that substance P infusion changed the spatial distribution of locomotion and rearing. The activity in the centre area in saline sessions was 7% of that in the periphery for both locomotion and rearing, whereas this was increased to 20% during the substance P session.

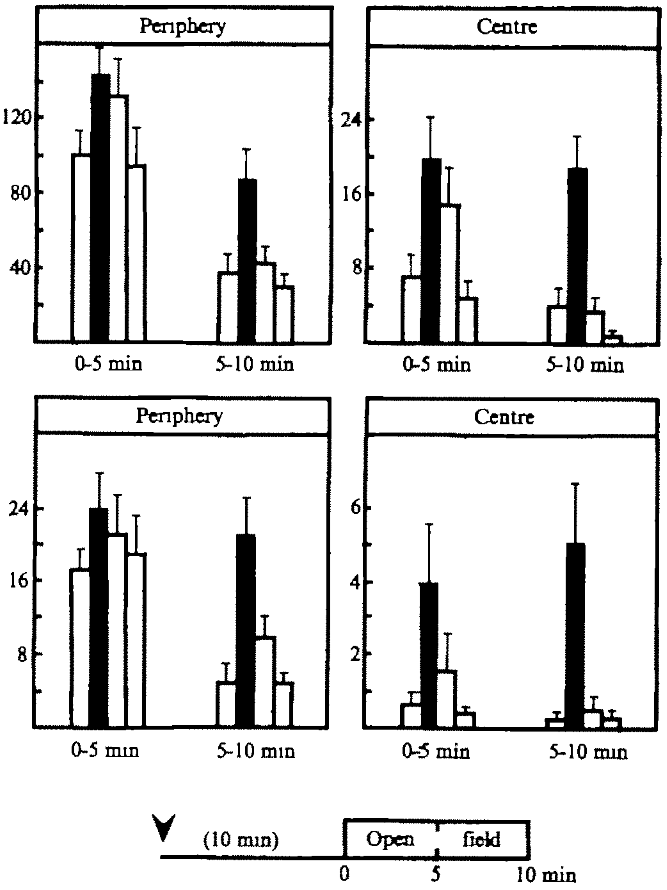
The examination of the drug × time interaction revealed a significant difference in the evolution of rearing between saline and SP sessions (rearing periphery $F = 2.4$, d.f. = 3,30. $P < 0.05$; rearing centre, $F = 4.4$, d.f. = 3,30, $P < 0.01$). This implies that although habituation was observed in saline sessions, during the substance P session rats remained activated throughout the 10 min. Individual comparisons between mean rearing scores on the last 5 min indicated a significant difference between substance P and all saline sessions (Newman-Keuls, $P < 0.01$). Twenty-four hours after the substance P injection motor behaviours in the open field were still elevated above control levels for the first 5 min of the session; but only the increased locomotion at the periphery was significant ($P < 0.01$).

d-Amphetamine-induced locomotor activation is mediated by DA-A10 neurones[15], and the magnitude of the response to *d*-amphetamine is determined by the firing rate of these neurones[16]. To evaluate a possible activation of DA-A10 neurones by substance P, we tested the reaction of 5 rats in photocell cages to a low dose of *d*-amphetamine (0.5 mg per kg i.p.) 1 h after bilateral saline (1 μl) of substance P (3 μg) infusion into the VTA. Four days elapsed between these two test sessions. Substance P significantly potentiated the effect of *d*-amphetamine (activity counts during 1 h after *d*-amphetamine: saline session 333 ± 123, substance P session 1,503 ± 473, $P < 0.001$). The magnitude of this effect cannot be interpreted by the simple addition of the residual substance P effect and the *d*-amphetamine effect, since it is five times that observed after saline infusion.

One problem encountered with intracerebral infusions is that of diffusion to neighbouring structures; it is possible that the substantia nigra (SN), which contains a high amount of substance P, was affected by our injections. We have previously reported, however, that similar injections of substance P into the SN at a point 1.5 mm lateral to the VTA site elicit a syndrome of a quite different nature; consisting of stereotyped movements and, in particular, considerable self-grooming[17]. Infusion of substance P into the VTA induces a behavioural activation characterised by increased exploratory behaviour. An apparent discrepancy to note is that one consequence of lesions of the DA-A10 neurones is a permanent hyperlocomotion[18]. This particular hyperlocomotion, however, is associated with decreased exploration and a disruption of goal-directed behaviour[19]. The activation induced by substance P is interpreted as an enhancement of purposeful and organised behaviours; lesion-induced hyperlocomotion could reflect an inability to attend or to 'focalise' behaviour.

It is possible that the potentiation of the *d*-amphetamine effect after infusion of substance P into the VTA results from increased firing rate of DA-A10 neurones. This is consistent with the ipsilateral increased release of newly synthesised ^{3}H-DA observed in striatum by Cheramy *et al.*[10], after application of substance P into the substantia nigra in the cat. Moreover, the *d*-amphetamine results indicate a long-lasting effect of substance P on DA-A10 neurones. Iontophoretic application of substance P in areas rich in substance P innervation shows a predominant excitation response with slow onset and long duration which is still present between 6 and 15 min after the end of the application[7]. Thus it is possible that VTA substance P-containing neurones, which originate principally in the medial habenular nucleus, exert a tonic activation on DA-A10 neurones. This hypothesis is strengthened by our recent results in which substance P-induced behavioural activation was

Fig. 2 Effects of acute bilateral infusion into the ventral tegmental area of isotonic saline or substance P on locomotion and rearing activities in the open field. Open bars indicate the saline test sessions (first, third and fourth days) and solid bars indicate the SP test session (second day). The locomotion (upper) histograms show the number of squares entered per 5-min period. The rearing (lower) histograms show the number of rears per 5-min period. The peripheral area was the band of squares adjacent to the walls; the centre area consisted of the inner squares. The arrow indicates that infusions were made 10 min befor testing. The open field was 100 cm × 100 cm × 70 cm; the floor was divided into 20-cm squares and locomotion was measured by the number of squares entered. The central area was 60 cm × 60 cm. The rats were transported by hand and placed in the centre of the open field.



blocked by local application of haloperidol in the DA-A10 terminal areas, and by 6-hydroxydopamine-induced lesion of these terminals. Precisely which specific structures are involved in mediating the response to substance P cannot yet be determined. The medial habenular nucleus receives strong afferents from limbic structures such as septum, diagonal band of Broca and nucleus accumbens[20–22], all of which are innervated by DA-A10 neurones. The habenular nucleus also receives dopaminergic input from the VTA[23] and serotonergic afferents from the raphe nuclei[24] which are implicated in the control of DA activity[25,26]. It is proposed that the substance P innervation of the VTA is part of a descending feedback loop which modulates the mesolimbic and mesocortical dopaminergic systems tonically.

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Denervation supersensitivity in the striatonigral GABA pathway

POSTSYNAPTIC receptors deprived of their associated presynaptic terminals exhibit denervation supersensitivity by showing enhanced responses to agonist drugs and elevated binding of radiolabelled ligands[1–4]. To investigate whether this effect also occurs in γ-aminobutyric acid (GABA) neurones we have denervated the receptors for a well defined GABAergic projection in the rat brain, using the kainic acid lesion technique, and have found them to show enhanced responses to a GABA agonist drug and correlated elevations in radioligand binding.

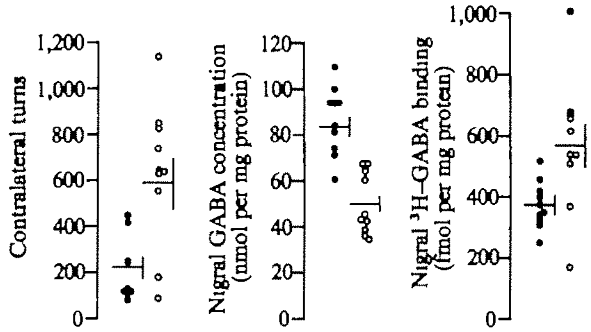

Fig. 1 Left: rotational responses in automated rotometers[20] 1 h after unilateral intranigral injection of muscimol (2 ng; stereotaxic coordinates: A2.2, V-2.8, L2.0, König & Klippel[29]) made 3 weeks after ipsilateral striatal KA lesions (2.5 μg; coordinates A8.0, V0.0, L2.5) or in unlesioned (control) rats. Intracerebral injections were made by slow-motion infusion in 1 μl saline, under light ether anaesthesia for muscimol or barbiturate anaesthesia for KA, as previously described[7,8,15]. Comparisons were by Mann–Whitney U test (2-tailed) ($P < 0.02$). Centre: nigral GABA concentrations in the substantia nigra of lesioned and unlesioned (control) hemispheres of rats 4 weeks after unilateral striatal KA lesions, determined by radioreceptor assay[22]. Comparison by Wilcoxon T test (2-tailed) ($P < 0.01$). Right: specific Na^+-independent high-affinity ^3H-GABA-receptor binding in Triton-treated membrane preparations of substantia nigra from lesioned and unlesioned (control) hemispheres of rats 4 weeks after unilateral striatal KA lesions, using ^3H-GABA at 10 nM (ref. 21). Specific binding was defined as the difference between binding occurring in the presence and absence of an excess (100 μM) of unlabelled GABA. Comparison by Wilcoxon T test (2-tailed) ($P < 0.03$). Protein contents of nigrae dissected from lesioned (116.5±6.5 μg) and unlesioned (113.5±9.5 μg) hemispheres were indistinguishable. ● Control; ○ lesion.

These results indicate that GABA receptors can show denervation supersensitivity and suggest that elevated GABA-receptor binding in Huntington's chorea may be of functional significance for the pharmacotherapy of this disorder.

Using the Ungerstedt rotating rat model[5], in which the nigrostriatal dopamine system in one hemisphere is destroyed by intracerebral injection of the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA), supersensitivity of denervated striatal dopamine receptors is manifested by a contralateral rotational response to peripheral administration of the dopamine-receptor agonist apomorphine. This has been shown to be associated with elevated binding of the dopamine-receptor ligands ^3H-haloperidol[3] and ^3H-spiperone[4], which correlates with the degree of apomorphine-induced turning[4]. The unilaterally 6-OHDA-lesioned rat has provided a useful animal model for the study of the physiology and pharmacology of dopaminergic mechanisms[2–6]. As GABA-dependent, dopamine-independent, contralateral rotational behaviour results from unilateral intranigral injection of GABAergic drugs[7–12], we sought to define a unilaterally GABA-lesioned model to facilitate the study of the physiology and pharmacology of GABAergic mechanisms in an analogous manner to the unilaterally 6-OHDA-lesioned rat. Thus, we destroyed the cell bodies of origin of the striatonigral GABA pathway[13,14] and examined rotational responses to a GABAmimetic drug and ^3H-GABA receptor binding in the putatively denervated nigra.

Male Sprague-Dawley rats, 150–200 g, were given unilateral stereotaxic injections of kainic acid (KA, 2.5 μg in 1 μl saline) to destroy striatal perikarya, including the cell bodies of origin of the striatonigral GABA pathway[16,17]. After 21 d rats were given unilateral intranigral injections of the potent and specific GABA agonist muscimol[18,19] (2 ng 1 μl^{-1}) ipsilateral to the KA lesion, and resulting rotational behaviour was quantified in automated rotometers[20]. After a further 7 d rats were decapitated and nigral tissue dissected from each hemisphere from frozen sections. Specific ^3H-GABA binding in Triton-treated membrane preparations of each nigra was determined using

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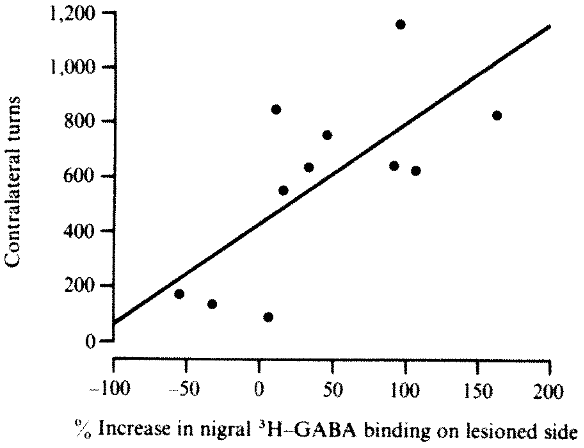
^{3}H-GABA at 10 nM (ref. 21), and nigral GABA levels were determined using a sensitive radioreceptor assay[22].

Unlesioned control rats showed contralateral rotation to this low dose of muscimol administered unilaterally into the substantia nigra[7–11]. In successfully lesioned rats an enhanced rotation was seen when muscimol was administered into the denervated nigra (+173%, $P<0.02$). In these rats, ^{3}H-GABA binding was greater in the nigra of the lesioned hemisphere than in that of the contralateral unlesioned hemisphere (+52%, $P<0.03$), and GABA levels were reduced (−44%, $P<0.01$) (Fig. 1). In lesioned animals there was a significant correlation between rotational response to intranigrally administered muscimol and elevation of nigral ^{3}H-GABA binding ($r=0.686$, $P<0.05$) (Fig. 2).

A Scatchard analysis of saturation data, carried out on the nigra of lesioned and unlesioned hemispheres, revealed that the elevation of ^{3}H-GABA binding in the nigra of the KA-lesioned hemisphere was primarily characterised by an 81% increase in maximal high-affinity ^{3}H-GABA binding (B_{max}), with no change in dissociation constant (K_D). B_{max} for low-affinity ^{3}H-GABA binding was elevated by 37% in the lesioned nigral preparations, with no change in K_D (Fig. 3). This profile of a denervation-induced increase in GABA-receptor sites, without a change in affinity for their ligand, is qualitatively similar to that seen in the dopamine-denervated striatum using ^{3}H-haloperidol as a dopamine-receptor ligand[3].

Rats with denervated nigral GABA receptors showed an enhanced rotational response to the GABA agonist muscimol and elevated nigral binding of ^{3}H-GABA; rotational responses and elevation of ^{3}H-GABA binding were significantly correlated. These findings are consistent with the development of denervation supersensitivity of nigral GABA receptors deprived of their associated presynaptic terminals by destruction of their cell bodies of origin. This GABA-dependent rotational behaviour has been shown to be independent of nigrostriatal dopaminergic mechanisms[7–12], although these effects of GABA agonists may mimic those of dopamine agonists in the 6-OHDA-lesioned rat (that is, induce contralateral rotation) by acting beyond the striatum, at the level of the substantia nigra; the striatonigral pathway has been shown to constitute the striatal output system[23,24]. We therefore propose that our study demonstrates a GABA-dependent rotational behaviour model in which GABAergic parameters, including correlated behavioural and receptor-binding indices of supersensitivity after unilateral lesions of descending GABA pathways, are similar in many respects to their equivalent dopaminergic parameters in the dopamine-dependent rotational model[2–4].

Fig. 2 Correlation between rotational response 1 h after 2 ng muscimol, administered unilaterally into the substantia nigra and elevation of nigral ^{3}H-GABA-receptor binding, after ipsilateral striatal KA lesions. Significant correlation: $r=0.686$, $P<0.05$ (2-tailed). Line of best fit indicates a value of rotation ($\simeq 400$ turns h^{-1}) for zero change in binding that is within the control range of turning for this dose of muscimol administered unilaterally into the substantia nigra of unlesioned rats (see Fig. 1).



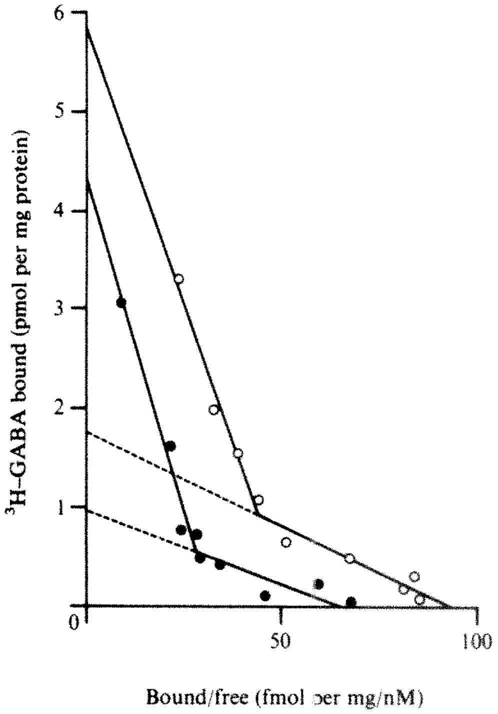


Fig. 3 Scatchard analysis of saturation data from ^{3}H-GABA-receptor binding carried out as described in Fig. 1 using 1–100 nM ^{3}H-GABA, on substantia nigra from lesioned and unlesioned (control) hemispheres of rats with unilateral striatal KA lesions. Maximum binding (B_{max}, fmol per mg protein) and dissociation constant (K_D, nM) for high-affinity ^{3}H-GABA binding were 958 and 15, respectively, in control animals (●), and 1,735 and 18, respectively, in lesioned animals (○). Respective B_{max} and K_D values for low-affinity ^{3}H-GABA binding were 4,234 and 130 in controls, and 5,831 and 112 in lesioned animals. Results are from pooled tissue of a further 6 lesioned animals.

The results demonstrate for the first time a correlation between receptor supersensitivity as indexed by a behavioural test and a ligand binding technique in a non-dopaminergic system. This model may be useful for studying the physiology and pharmacology of GABAergic mechanisms.

In addition, the striatal KA lesion has been proposed as a biochemical[16,17] and behavioural[25] model for Huntington's chorea. Our demonstration of GABA supersensitivity in the substantia nigra after such lesions provides additional support for the validity of this model, as the substantia nigra in post-mortem brains from choreic patients shows elevated ^{3}H-GABA binding[26]. Our study also suggests that, contrary to previous suggestions[27], GABA agonists may be of limited therapeutic potential in this condition. By acting on such supersensitive nigral GABA receptors at the level of the striatal output system, they may mimic those effects of increased dopaminergic transmission that are thought to be detrimental to clinical state in Huntington's chorea[28].

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Calcium-dependent exocytosis in bovine adrenal medullary cells with leaky plasma membranes

SECRETION from nerve terminals and many other cell types occurs by exocytosis, a process in which the contents of small intracellular vesicles are released after fusion of the vesicle membrane with the plasma membrane of the cell. Exocytosis generally requires calcium ions[1,2], but little is known about the site at which these Ca ions act. The electrophysiological experiments of Katz and Miledi[3] strongly point to an intracellular site, a conclusion that is supported both by the observation that transmitter release is increased after microinjection of Ca into the presynaptic terminal of the squid giant synapse[4] and the finding that the Ca ionophore A23187 promotes secretion from a variety of cells[5]. Further progress has been severely hampered by lack of preparations in which this presumed intracellular site is freely accessible to experimental investigation. We now present evidence that the plasma membrane of medullary cells from the bovine adrenal gland can be rendered permeable to small molecular weight substances without blocking Ca-dependent exocytosis. This preparation has been used to examine the dependence of exocytosis on Ca and Mg ions.

Cells were obtained from thin slices of bovine adrenal medulla by protease digestion[6]. Catecholamine can be released in a Ca-dependent manner by stimulation with carbamylcholine, veratridine or potassium chloride. The Ca-dependence of catecholamine release in response to these secretagogues is shown in Fig. 1. In all cases secretion is half-maximal at Ca concentrations between 0.1 and 1 mM, and is associated with the release of the vesicular enzyme dopamine-β-hydroxylase but note cytosolic enzyme lactate dehydrogenase. Secretion is inhibited by the Ca channel blocker D600 (10^{-4} M).

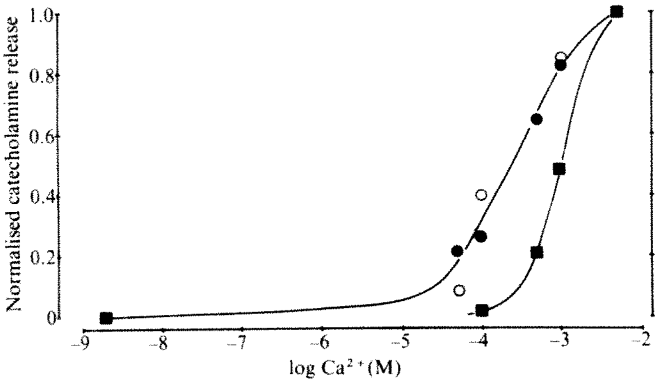

Fig. 1 Ca-dependence of catecholamine release from intact bovine adrenal medullary cells in response to 5×10^{-4} M carbamyl choline (●), 5×10^{-4} M veratridine (■) and to a potassium challenge (○). Ordinate: catecholamine release normalised to that in 5 mM Ca. Catecholamine was assayed fluorimetrically by the trihydroxyindole method[7]. Incubation was for 15 min at 37 °C after which time the cells were removed by centrifugation and samples of the supernatant taken for analysis. The medium contained (mM) NaCl, 136; KCl, 2.7; glucose, 5; Mg, 2; HEPES, 16 *p*H 7.2 and for the potassium challenge NaCl, 68; KCl, 70.7; glucose, 5; Mg, 2; HEPES, 16 *p*H 7.2. The media containing 2×10^{-9} M Ca^{2+} (calculated) contained in addition 5 mM EGTA.

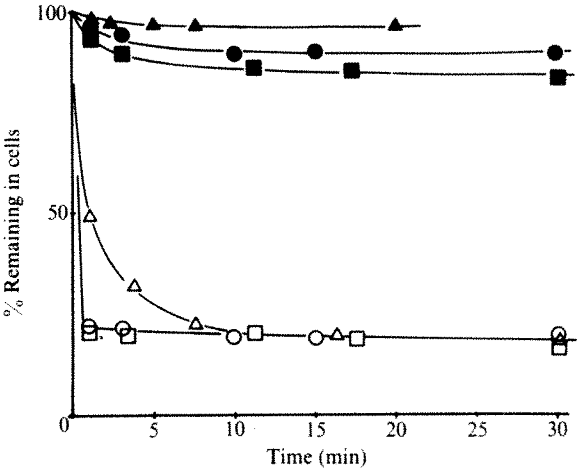

Fig. 2 Release of ^{86}Rb and catecholamine after exposure to high-voltage discharges. 1 ml of a suspension of medullary cells (10^6 per ml) preloaded with ^{86}Rb was placed between a pair of stainless steel electrodes (0.9 cm apart and each 1.0 cm^2) in a square perspex container and subjected to the discharge of a 2 μF capacitor. Cells exposed at zero time to 1 (▲, △), 5 (●, ○) and 10 (■, □) discharges, τ approx 200 μs. Field 2 kV cm^{-1}. Medium (mM): potassium glutamate, 138.7; glucose, 5; Mg, 2; PIPES, 20 *p*H 6.6; Ca-EGTA buffer, 5. Calculated free Ca^{2+} 10^{-5}M. Temperature 37 °C. Closed symbols represent catecholamine release and open symbols ^{86}Rb release. Similar results were obtained with cells preloaded with the non-metabolisable sugar 3-*O*-[^{14}C]methyl-D-glucose showing that the increase in permeability is not restricted to charged molecules.

To provide ready access to the cell interior, suspensions of cells were exposed to a small number of high-voltage discharges[8]. This technique was used previously to alter the intracellular environment of erythrocytes[9,10]. It presumably works by causing localised dielectric breakdown generating holes in the plasma membrane. This increase in permeability is most readily demonstrated in cells preloaded with ^{86}Rb where a suitable shocking regime can release 80% of the cellular ^{86}Rb within a few minutes (Fig. 2). As we wished to gain ready access to the cell interior for Ca-EGTA buffers, we also examined the uptake of ^{51}Cr-EDTA. In conditions similar to those used in Fig. 2, the ^{51}Cr-EDTA space increased within 5 min to a new steady level that varied between 50 and 75% of the cell volume. This suggests that the cells should have also been rendered freely permeable to Ca-EGTA which is only a little larger than Cr-EDTA. Although erythrocytes reseal after exposure to high voltage discharges, this does not seem to happen in adrenal cells where measurements of the ^{51}Cr-EDTA space show that the cells remain freely permeable for at least 20 min at 37 °C.

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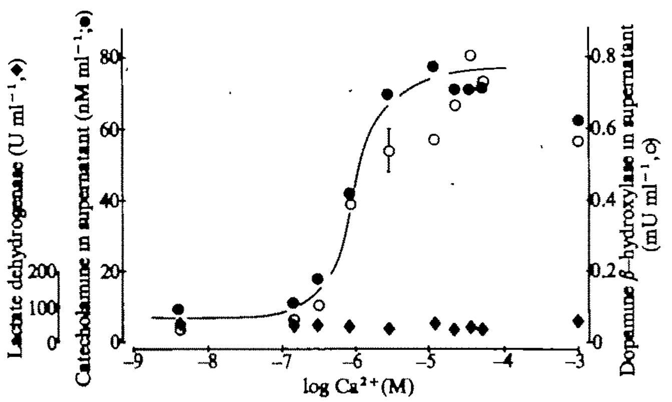

Fig. 3 Ca-dependence of catecholamine (●); dopamine-β-hydroxylase (○), and lactate dehydrogenase (◆) release in response to five discharges (2 kV cm^{-1}, $\tau \sim 200$ μs) over about 30 s. The samples were centrifuged and assayed 15 min after the discharges. Medium (mM): potassium glutamate, 138.7; glucose, 5; Mg^{2+} 2; PIPES, 30; Ca-EGTA buffer, 5; *p*H 6.6. Temperature 37 °C. Catecholamine was assayed fluorimetrically[7]. Dopamine-β-hydroxylase was assayed spectrophotometrically by measuring conversion of tyramine to octopamine[11]. Lactate dehydrogenase was assayed spectrophotometrically[12].

When cells are exposed to high-voltage discharges in media containing Ca, catecholamine is released (Fig. 2). This release is Ca-dependent, but is not mediated by depolarisation nor does it involve Ca channels. Release is not brought about by the temperature jump generated by the high-voltage discharge. Less than 1% of the total intracellular catecholamine is normally released after exposure to high-voltage discharges in nominally Ca-free media containing EGTA though the cells are rendered freely permeable to Cr-EDTA. With calcium present more than 10% of the total catecholamine is released and this response persists unchanged in cells fully depolarised by potassium and in the presence of a Ca channel blocker D600 (10^{-4} M). This Ca-dependent release of catecholamine seems to reflect exocytosis because it is associated with the selective release of the vesicular enzyme dopamine-β-hydroxylase but not lactate dehydrogenase which is present in the cytosol and is very temperature-dependent, being reduced by over 90% when the temperature is lowered from 37 °C to 5 °C. The catecholamine release is unlikely to result from a direct effect on the vesicles because exposure of isolated chromaffin granules to the same shock regime gives no release of catecholamine or dopamine-β-hydroxylase.

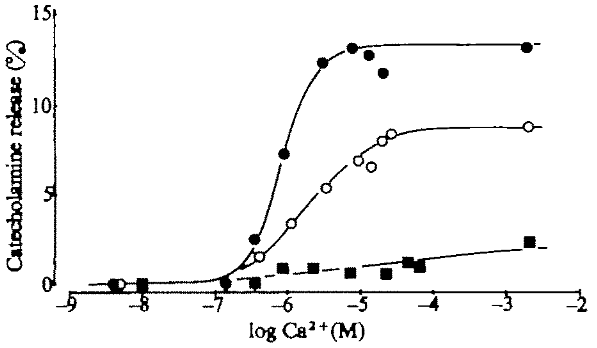

Fig. 4 Ca-dependence of catecholamine release at different magnesium levels in response to five discharges of 2 kV cm^{-1}. The samples were centrifuged and assayed 15 min after the discharges. Medium (mM): potassium glutamate, 138.7; glucose, 5; PIPES 20; Ca-EGTA buffer, 10.4, *p*H 6.6; $MgCl_2$ 2, (●); 10, (○); 50, (■). Temperature 37 °C. The time constant of the discharge is dependent on the conductivity of the medium and would have been close to 200 μs for media containing 2 mM $Mg\ Cl_2$ and 140 μs for media containing 50 mM $Mg\ Cl_2$.

Working with cells rendered permeable by exposure to high-voltage discharges, we have investigated the dependence of exocytosis on the level of ionised Ca and Mg in the medium in which the shocks are delivered. Using two Ca-buffers, EGTA (Fig. 3) and EDTA, the Ca concentration giving half-maximal activation of the release of both catecholamine and dopamine-β-hydroxylase is close to 1 μM in the presence of 2 mM Mg. This is more than two orders of magnitude lower than the Ca concentration required for half-maximal activation of secretion from intact cells (Fig. 1), providing further evidence that exposure to high voltage discharges renders the interior of the cell freely accessible to externally applied Ca-buffers. Figure 4 shows that raising the Mg concentration both reduces the apparent affinity for Ca and also leads to a decrease in the total amount of catecholamine released.

Another interesting feature of Figs 3 and 4 is that only about 10% of the total cellular catecholamine and dopamine-β-hydroxylase is released even though the cells are freely permeable to Cr-EDTA. At first sight this is very surprising as one might expect that if Ca has free access to an intracellular site controlling exocytosis all the vesicular catecholamine and dopamine-β-hydroxylase should ultimately be released. There may, however, be only a limited number of release sites and the conditions used in our experiments may not permit repriming these sites for more than one round of exocytosis.

In all these experiments cells have been suspended in the test medium and then exposed to a series of high-voltage discharges. This means that the site at which Ca acts is still exposed to a fairly normal intracellular environment. In order to probe further the ionic and metabolic requirements for exocytosis it is necessary to render cells freely permeable in the absence of Ca and only expose them to Ca after adequate time has been allowed for small molecular weight materials to equilibrate. Figure 5 shows that this is technically possible. Preliminary results indicate that after exposure at 37 °C for longer than 5 min to Ca-free solutions lacking ATP, addition of Ca fails to promote exocytosis whereas Ca remains fully effective for at least 30 min provided ATP is present throughout the period of exposure to nominally Ca-free media. These results suggest that ATP or something derived from it is necessary for Ca-dependent exocytosis in adrenal medulla cells. A similar conclusion had been reached from experiments on intact cells[13,14], but the present preparation seems to offer an excellent opportunity to discover the part played by ATP in exocytosis. Recently there have been a number of claims that Ca-dependent exocytosis can occur in cell-free systems[15–17]. A feature common to these systems and the present preparation is that all are activated by micromolar

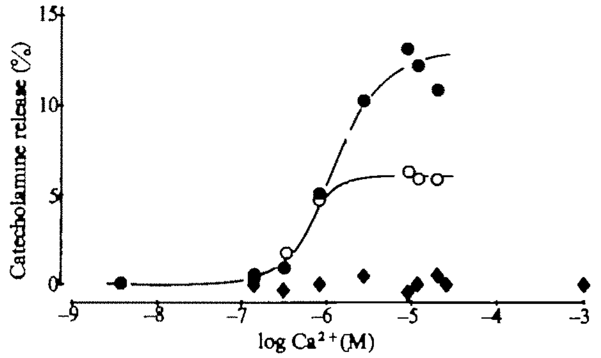

Fig. 5 Influence of ATP on Ca-dependent catecholamine release. Ten discharges 2 kV cm^{-1}, $\tau \sim 200$ μs were applied to cells in a medium of (mM): potassium glutamate, 138.7; glucose, 5; PIPES, 20; EGTA, 0.4; ATP, 0 (◆), 1 (○), 5 (●); *p*H 6.6. The cells were incubated for 30 min at 37 °C and then Ca-EGTA buffer at *p*H 6.6 was added to give a final buffer concentration of 10 4 mM. After a further 15 min at 37 °C the samples were centrifuged and assayed.

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concentrations of Ca which seems well within the physiologically possible range[18]. It is also within the range in which troponin and related Ca-binding proteins are activated.

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Depolarising effect of curare on embryonic rat muscles

CURARE (*d*-tubocurarine) reduces the responsiveness of skeletal muscle fibres to nerve stimulation[1] by acting as a competitive antagonist to the transmitter acetylcholine (ACh)[2]. In adult muscle this action is not accompanied by any obvious change in muscle fibre resting potential, such as is produced by depolarising blocking agents[3]. We report here that in rat embryos curare evokes large depolarisations through action on ACh receptors.

Experiments were carried out on internal intercostal muscles from rat embryos (15–20 d gestation) and neonatal pups, with standard recording techniques[4]. During this developmental period intercostal muscles differentiate from primary myotubes to myofibres[5]. At all ages studied the muscle cells had resting membrane potentials of −70 to −90 mV in normal saline solution (see legend to Fig. 1), most displayed both spontaneous miniature endplate potentials (m.e.p.ps) and nerve-evoked endplate potentials (e.p.ps), and nerve stimulation produced contraction. When these preparations were perfused with medium containing 1–10 μM curare (Calbiochem; Sigma), the recorded resting potentials fell to about −50 mV (Table 1), m.e.p.ps were reduced in amplitude or absent, evoked e.p.ps were small and the muscles gave only a weak contraction in response to nerve stimulation. When the curare was washed out the resting potentials recovered gradually and e.p.ps and m.e.p.ps reappeared. In some cases, we recorded from individual myotubes continuously during the application and washout of curare. The membrane potential in these fell from about −80 mV to about −50 mV in 10–14 min and returned to normal 16–20 min after washing. Presumably, most of this time was required for equilibration of curare in the bath.

Fibres depolarised by bath-applied curare did not desensitise. Reduced resting potentials (~ −50 mV) were still recorded after muscles had been in 10 μM curare for 2 h or more. A similar absence of desensitisation has been reported for dissociated embryonic rat myotubes depolarised by ACh[6].

A depolarising response was also seen when curare was applied focally from a micropipette. Glass microelectrodes were broken to tip openings of 5–15 μm and filled with a 0.5 mM solution of curare in saline; this was ejected by applying 0.5–1 kg cm^{-2} pressure in pulses of 0.1–2 s duration. Curare application by this means frequently produced localised regions of contraction in embryonic muscle. Intracellular recording revealed that during focal curare application the fibres are transiently depolarised by as much as 35 mV (Fig. 1). Some responses exceeded threshold and gave rise to action potentials (Fig. 1*b*). The amplitude of the response elicited was graded with the duration and pressure of the pulse and with the proximity of the curare pipette to the recorded myofibre. The effect did not seem to depend on the action of curare on presynaptic nerve terminals[7], because (1) the depolarising response could be evoked by focal curare application anywhere along the length of the myotubes, (2) focal application did not increase the frequency of spontaneous m.e.p.ps before their abolition, and (3) the response was undiminished by the presence of 0.5 μg ml^{-1} tetrodotoxin in the bath. As a control against possible artefacts arising from the pressure application technique, 15 fibres were subjected to similar pressure pulses from pipettes containing saline solution alone; none responded with depolarisations like those seen with curare (in several cases rapid depolarisations or hyperpolarisations of 2–5 mV were produced by the pressure pulse).

Two lines of evidence indicated that the effect of curare results from action on ACh receptors with a consequent increase in ionic conductance, as occurs during normal neuromuscular transmission. First, the effect of curare was abolished by preincubating the muscle in saline solution containing α-bungarotoxin, which binds ACh receptors with high specificity[8,9]. Embryonic muscles (16 d) were incubated with 5 μg ml^{-1} toxin for 2 h, the unbound toxin was washed out and myotube resting potentials were sampled before and after adding 10 μM curare to the bath (Table 1). The curare had no effect on membrane potential after incubation in the toxin. Likewise, preincubation of muscles in the toxin prevented the phasic myofibre depolarisation and contraction evoked by focal application of curare (as in Fig. 1). Second, curare application reduced myofibre input resistance. Resistances were monitored by application of negative inward current pulses through the recording microelectrode, by means of a bridge circuit. After a single pulse of curare to the surface of myofibres, the input resistance dropped by up to 85% during the rising phase of the depolarisation (Fig. 1), and then gradually returned to its original value, 20–50 MΩ. In contrast, no comparable changes in resistance were detected when the fibres were depolarised (by as much as

Table 1 Membrane potentials recorded in various conditions from rat muscle fibres

	Membrane potential (mV)		
Age	Saline	Saline + curare	Saline + curare (preincubated in α-bungarotoxin)
15–16 d gestation	75.6 ± 0.9 (120/20)	48.3 ± 2.4 (21/3)	76.7 ± 1.7 (18/2)
17–19 d gestation	76.7 ± 0.6 (164/16)	47.0 ± 0.5 (130/15)	
0–3 d postpartum	70.1 ± 1.0 (52/8)	51.1 ± 0.8 (53/5)	
5 d–4 wk postpartum	63.6 ± 1.0 (18/2)	65.8 ± 0.6 (190/10)	
Innervated adult	84.3 ± 0.8 (35/3)		
Denervated adult	62.7 ± 0.9 (42/3)	64.8 ± 0.8 (56/3)	

The values indicate mean ± s.e.m. (no. of fibres per no. of muscles). Curare concentrations were 1–10 μM when applied to embryonic and neonatal muscles, and 0.5 mM when applied to denervated muscles. Curare was applied 20–120 min before potentials were recorded. Muscles preincubated in α-bungarotoxin were exposed to 5 μg ml^{-1} for 2 h.

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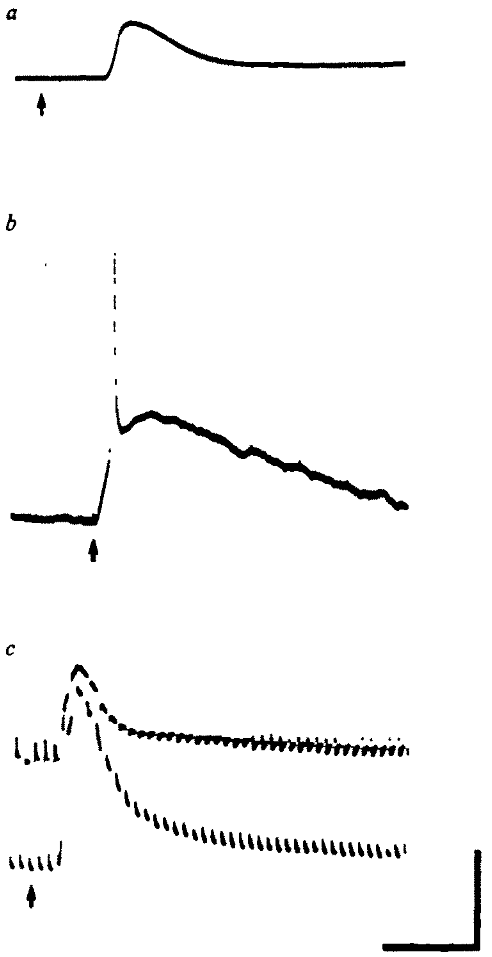

Fig. 1 Muscle fibre depolarisations evoked by focal curare application. Responses from different fibres, without (*a*, *c*) and with (*b*) an accompanying action potential. Curare was applied at the times indicated by arrows by the method described in the text. In *c*, fibre input resistance was monitored synchronously by injecting hyperpolarising current pulses of 50-ms duration and 1-nA amplitude at 300-ms intervals; the amplitude of the potential changes evoked by the current pulses was proportional to membrane resistance. It can be seen that the resistance decreased by 85% in this fibre during the curare response. Note that only partial repolarisations occurred during the intervals illustrated; characteristically, full recovery of the resting potential occurred within one or a few minutes of local curare application. Vertical calibration, 40 mV in *a* and *c*, 10 mV in *b*. Horizontal calibration, 4 s in *a* and *c*, 10 s in *b*. All experiments were done at room temperature (18–21 °C) in oxygenated saline containing 1–5% calf serum and (in mM): NaCl, 118; KCl, 3.0; $CaCl_2$, 4.0; $MgCl_2$, 1.1; glucose, 5.5; HEPES, 4.2 (*p*H 7.2).

50 mV) by raising external potassium to 50 mM through substitution for sodium. These results suggest that the curare acts on ACh receptors to produce an increase in membrane permeability.

The sensitivity of embryonic muscle fibres to curare disappeared at about the time of birth (21–22 d gestation). Of eight muscles examined 0–3 d postpartum, five were depolarised by 10 μM curare in the bath and three were not. The phenomenon was not observed in 10 muscles examined ≥5 d after birth.

As neonatal rat ACh receptors have some properties in common with extrajunctional receptors of denervated adult muscle[10–12], we considered the possibility that adult extrajunctional receptors might also be depolarised by curare. We denervated intercostal muscles in three adult rats and one week later confirmed that they were supersensitive to focally applied carbamylcholine. However, none of the fibres sampled in these muscles was depolarised by 0.5 mM bath-applied curare (Table 1), and none of 10 fibres was depolarised by focally applied 5 mM curare. This agrees with previous studies of the blocking action of curare on denervated rat diaphragm[13,14], in which no depolarising action was observed. By this criterion, then, the receptors of embryonic muscle differ from extrajunctional receptors of adult muscle.

Thus a depolarising response to curare seems to occur only in embryonic muscle fibres. One interpretation of this action is that a third type of ACh receptor exists on mammalian muscle, in addition to the well known junctional and extrajunctional receptors. Ritchie and Fambrough[15] previously suggested that the "molecular identity of the receptors" on rat myotubes may differ from those on normal and denervated adult muscle, on the basis of reversal potentials for ACh-evoked responses. An alternative interpretation, which does not assume a new class of receptors, is that some feature of the membrane environment, such as fluidity, changes in a manner such that the same curare–receptor interaction initially has a channel-opening effect and later does not. Whatever the modification involved in the loss of responsiveness to curare, it must occur soon after birth. It is interesting that one week after birth there is also a marked shift in the gating properties of ACh-activated conductance channels in rat muscle[16].

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Effect of tetraethylammonium on noradrenaline release from cat spleen treated with tetrodotoxin

EXPERIMENTS by Katz and Miledi[1] on the giant synapse of the squid stellate ganglion pretreated externally with tetrodotoxin (TTX) to block the conducted nerve impulses, and internally with tetraethylammonium (TEA) to block the potassium current, showed that a local regenerative response confined to the presynaptic terminal was obtained on depolarisation, provided that the external calcium concentration was sufficiently high. Strontium and barium substituted for calcium, and manganese and magnesium reduced the regenerative response. TEA, in the presence of TTX, also caused a massive release of acetylcholine from the frog neuromuscular junction in response to a depolarisation of the motoneurone terminals[2]. These results offered direct evidence for the existence in the nerve terminals of a TTX-resistant calcium channel involved in transmitter release, and suggested that TEA, by allowing an inward calcium

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Table 1 Effect of TEA on ^{3}H-NA release in TTX-treated spleen slices

	Fractional release per stimulus $\times 10^{2}$ (% of tissue ^{3}H-NA)				
	Krebs	TTX	TTX + TEA	TTX + TEA (calcium free)	TTX + TEA + lanthanum
^{3}H-NA	2.1 ± 0.4 (12)	0.3 ± 0.1 (12)	3.4 ± 0.7 (9)	0.2 ± 0.04 (9)	0.3 ± 0.1 (9)

All solutions contained phenoxybenzamine (1 µg ml^{-1}). ^{3}H-NA was released by applying field stimulation pulses for 20 or 50 s at 1 Hz. Release per stimulus is expressed as a percentage ($\times 10^{2}$) of tissue ^{3}H-NA content at the time of stimulation. Concentrations: TTX, 0.32 µM; TEA, 10 mM; lanthanum, 1 mM. Numbers in parentheses refer to the number of experiments.

current to become regenerative, enhanced transmitter release. The release of noradrenaline (NA) from sympathetic nerves also requires calcium, and further, strontium and barium substitute for calcium in sustaining release, and magnesium, manganese and lanthanum block release[3]. Moreover, TEA has also been shown to enhance NA release after electrical stimulation of sympathetic nerves[4–6]. These observations suggest that a calcium channel also exists in the sympathetic nerve terminals and is responsible for the release of NA. The present investigation was undertaken to determine if in the presence of TEA and TTX, depolarisation of the sympathetic nerve terminal initiates a 'regenerative' inward calcium current which leads to an explosive release of NA. We report that it does.

Cat spleen slices (1 mm thick) were incubated in ^{3}H-NA solution (16 ng ml^{-1}, specific activity 10.43 Ci mmol^{-1}) to label the endogenous stores of NA[7]. A slice was then transferred to a superfusion chamber which consisted of two halves of plexiglass with a hollowed-out chamber of about 1 ml capacity. Two chloride-coated silver-wire coils, arranged in parallel, served as electrodes and the slice was wedged between them. The open ends of the chamber held small stainless-steel tubes to a polyethylene tube, through which the superfusion fluid was introduced at the bottom and collected from the top. Superfusion was started with oxygenated Krebs–bicarbonate solution containing phenoxybenzamine (1 µg ml^{-1}) at 35 °C. The perfusion rate was about 8 ml min^{-1}. When spontaneous efflux of radioactivity had diminished to low and relatively constant levels, rectangular pulses (1 Hz, 20–80 V, 5 ms) were applied for 20 or 50 s. Effluent samples were collected for 2 min. Total tritium and ^{3}H-NA contents of the samples and the tissue were measured[8]. The increase in ^{3}H-NA release by nerve stimulation was obtained by subtracting the control background activity of the same volume of solution from the total output during stimulation. ^{3}H-NA release per stimulus is expressed as a percentage of the tissue radioactivity before stimulation.

Table 1 shows that ^{3}H-NA output due to field stimulation of sympathetic nerves was markedly reduced in the presence of TTX (0.32 µM). This blockade of release by TTX suggests that the field stimulation-induced release was mainly due to propagated action potentials. Residual release in the presence of TTX must be ascribed to the electrotonic depolarisation of the sympathetic nerve endings by current pulses applied by field stimulation, as in experiments in nerve terminals of the squid[9]. In the presence of TEA (10 mM), however, this residual response in the presence of TTX was restored to the control response obtained before TTX treatment. Secretory response in TEA plus TTX solutions occurred over a narrow range of voltages, and also at voltages much lower than those required to produce even a minimal response from preparations treated with TTX alone. This suggests that the release in the presence of TEA is not graded but may be triggered in a regenerative manner. Removal of calcium or addition of lanthanum (1 mM) abolished the response.

Our results show that in the presence of TEA, depolarisation of the sympathetic nerve terminals treated with TTX produced a massive release of ^{3}H-NA. The effect of TEA was restricted to nerve terminals, as conduction of nerve impulses was never restored at a time when explosive release from nerve terminals by local depolarisations could be demonstrated. Katz and Miledi[1,2] have previously discussed the possibility that inactivation of potassium efflux by TEA during depolarisation enables an inward calcium current to become regenerative. As TEA modifies NA release from sympathetic nerves in a manner comparable with its action on acetylcholine release from motoneurone terminals, it is possible that TEA may act similarly on the voltage-sensitive calcium channels in the sympathetic nerve terminals to facilitate calcium entry. We therefore suggest that one possible reason for the massive release of transmitter from TTX-treated preparations is the regenerative entry of calcium ions induced by the action of TEA on the sympathetic nerve terminals.

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Cell adhesion mediated by glycolipids

ADHESION between adjoining cells is a fundamental biological event, preceding both morphogenesis and organogenesis and also providing the basis for cellular recognition and membrane fusion[1–4]. Adhesion is apparently mediated by carbohydrate moieties on the plasma membrane as shown in studies of sugar transferases[5–7] and glycosidases[8]. A number of mechanisms such as the antigen–antibody reaction[9,10], the specific sugar transferase–substrate complex formation[11] and hydrogen bonding[11] between closely opposing carbohydrate chains have been proposed for the specific and nonspecific adhesion of cells, but the relevant surface molecules have not yet been isolated or identified. Two types of cell adhesion have been described: one is the reaggregation of dissociated animal cells in serum which may involve antibodies[9,10] or unidentified components of serum such as the conglutinin[12]. A second type of adhesion occurs in the absence of serum, and is probably regulated by glycoprotein factors[13,14]. Recently we found that lipid particles containing phospholipids or glycolipids were agglutinated by serum and that glycolipid particles adhered most strongly to tissue culture cells even in the absence of serum[15]. We report here that lipids, in particular glycolipids, could mediate adhesion of animal cells.

To determine which particular glycolipids can mediate adhesion, a model system was used involving lipid particles and monolayers of tissue culture cells as adhesion partners. Glycolipids, either prepared by chemical synthesis or isolated from natural sources, were incorporated into lipid particles and tested for adhesiveness on various tissue culture cells (for experimental details see legends to Table 1 and Fig. 2). These experiments

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showed that the lipid particles adhered most strongly to HeLa cells and less strongly to other cells in the following decreasing order: BHK > MDCK > 3T3 > MDBK > chick fibroblasts. These results suggest that the various cells contain differing numbers of combining sites in their plasma membranes. As the lipid particles were found to adhere most strongly to HeLa cells, these cells were used to test which lipids most effectively mediated adhesion. To measure the extent of adhesion, fluorescent lipids[16], either dansylcerebroside or dansylsphingosine, were used. The relative adhesiveness of the different lipid particles, measured by the intensity of their fluorescence, is summarised in Table 1. Comparable results were obtained when adhesion was estimated using a UV microscope. It can be concluded from Table 1, that although all lipid particles tested adhered to HeLa cells to varying extents, those particles containing galactose at the terminal positions were the most adhesive. Glycolipids with glucose, fucose, galactosamine or neuraminic acid at their terminal positions were less adhesive to monolayers. The galactose specificity is in accordance with the results of Chipowsky *et al.*[7] and Weigel *et al.*[18] who showed that 3T3 cells could most efficiently adhere to agarose beads which contained galactose as the ligand. Furthermore, the present experiments showed that adhesiveness increased with growing chain length of the sugar moiety. Glycolipids possessing four sugar molecules were the most adhesive of those tested—an exception was sulphatide, which is very adhesive although containing only one galactose molecule. The negative charge imparted by the sulphate group of the sulphatide does not seem to be the direct cause of its strong adhesion capacity, as other negatively charged lipids,

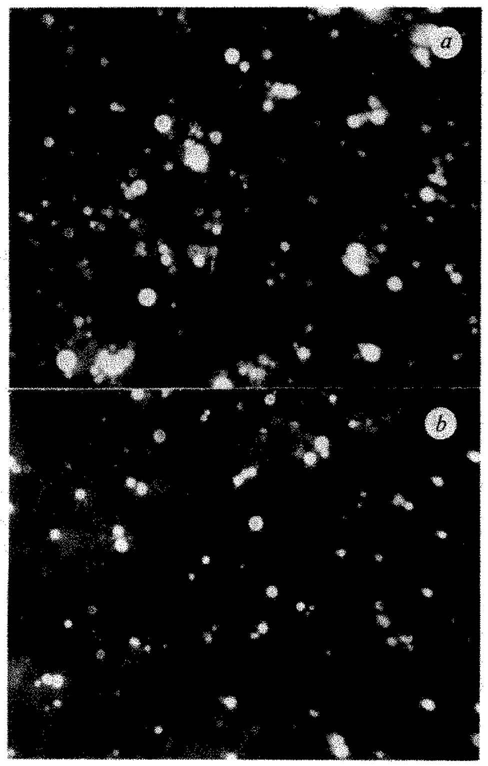

Fig. 1 Adhesion of glycolipid (ceramide-tetraoside, Table 1, compound *L*) particles to monlayers of HeLa cells and BHK cells. *a*, Adhesion to HeLa cells. *b*, Less extensive adhesion to BHK cells. To study adhesion, monolayers of tissue culture cells grown in Serva medium PM 13 to confluency in 5-cm diameter Petri dishes were switched to 2.5 ml of Dulbecco's medium[30] without serum. Aliquots of 0.1 ml of a suspension of glycolipid particles in PBS containing about 10^6 particles were added and the dishes were incubated for 2 h at 37° C. After the incubation period, the medium was decanted and the dishes were rinsed four times with PBS and observed using a UV microscope (Leitz).

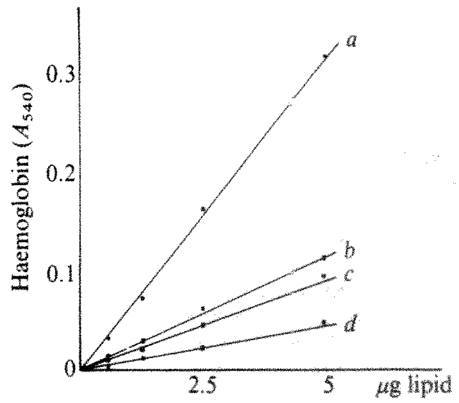

Fig. 2 Effect of glycolipids on the adhesivesness of erythrocytes to HeLa cells. *a*, Ceramide-tetraoside; *b* oligoglucoside; *c*, sphingomyelin; *d*, phosphatidyl inositol. Most lipids listed in Table 1 could be dissolved or finely emulsified in PBS by ultrasonic treatment. In the case of ceramide monohexoside, solubilisation was aided by addition of Tween 20 at a detergent to lipid ratio of 1:1. 100 μl of washed, neuraminidase-treated chick erythrocyte suspension containing 10 μl of packed cells was incubated at 37° C for 45 min with 100 μl of a PBS solution of lipid in the indicated amounts. After the incubation period, erythrocytes were washed three times with PBS. About 10^6 treated erythrocytes were incubated with HeLa cells as described for lipid particles (Fig. 1). The amount of adhered erythrocytes was determined as described in the legend to Table 1. Neuraminidase treatment of erythrocytes enhanced the effect of glycolipids. The standard condition of 0–5 μg of lipid per 100 μl of incubation mixture and an incubation period of 45 min was chosen, as a much higher concentration (>20 μg per 100 μl) of lipid and a prolonged incubation period (>1.5 h) may cause some haemolysis with long-chain glycolipid-treated erythrocytes.

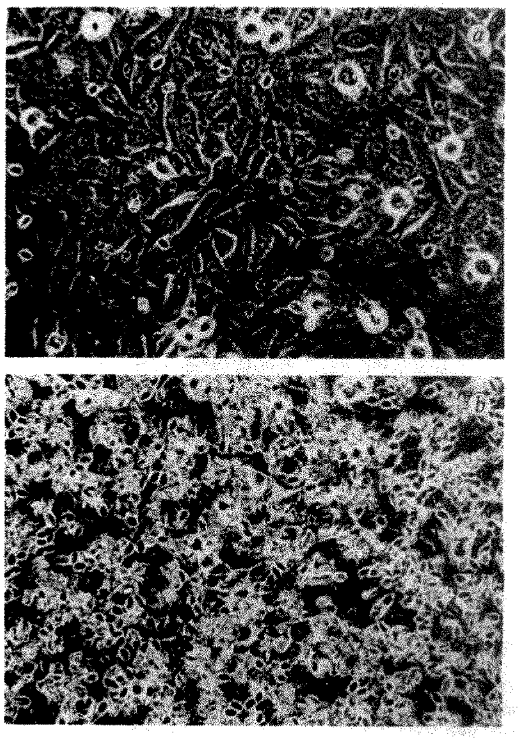

Fig. 3 Adhesion of erythrocytes to HeLa cells. *a*, Scant adhesion of neuraminidase treated erythrocytes to HeLa cells. *b*, Massive adhesion of neuraminidase treated erythrocytes, which have acquired a glycolipid (ceramide-tetraoside, *L* in Table 1) in their plasma membranes. Erythrocytes were incubated with 10 μl of ceramide-tetraoside in 100 μl of the incubation mixture for 45 min. Other methods as Fig. 2.

such as ganglioside or phosphatidyl inositol, did not adhere strongly. All the phospholipid particles tested adhered only moderately to HeLa cell monolayers. Nearly the same pattern of adhesion specificity with respect to the sugar moiety of glycolipids was obtained with BHK and MDCK cells. Adhesiveness of lipid particles to other cells was not rigorously quantitated.

To determine whether glycolipids could also promote adhesion between natural membranes, cells in which there was good correlation between adhesiveness and glycolipid content were required. Chick erythrocytes were satisfactory for this purpose, as they do not normally adhere to tissue culture cells but become artificially adhesive when glycolipids are incorporated into their membranes. The previously described method[16] for the incorporation of glycolipid into natural membranes was used. As shown in Fig. 2 adhesiveness of erythrocytes was enhanced on incubation with increasing lipid concentrations. Although the mechanism by which lipid is incorporated in this system is not known, we conclude from these results that lipids, especially glycolipids can promote the adhesion of natural membranes. Figure 3 demonstrates the difference in adhesiveness of glycolipid treated and untreated erythrocytes on HeLa cells. In a subsequent experiment all the lipids listed in Table 1 were incorporated into erythrocytes in identical conditions in order to determine their relative efficacy in enhancing adhesion of erythrocytes to tissue culture cells. Adhesion was measured by following the haemoglobin content which remained associated with the HeLa cells after washing. Results presented in Table 1 (right vertical column) correlated favourably with those obtained with lipid particles (left vertical column); that is, glycolipids containing at least four sugar molecules and a terminal galactose stimulated the adhesiveness of erythrocytes most. Comparable results were obtained from two independent experiments, so the values in Table 1 seem to represent the real relative adhesiveness of different lipids. Because of these results it also seems unlikely that the values shown for the glycolipid-coated erythrocytes reflected differential equilibrium in the binding of glycolipids to erythrocytes.

Glycolipids are specific components of plasma membranes[19]. It is known that animal cells contain a mixture of glycolipids, some of which terminate with a galactose molecule[20]. In the past, glycolipids have been considered to possess biological properties such as blood group specificity[20] or receptor functions[21,22], implying that they may be accessible to external factors. The present experiments have demonstrated a possible function of glycolipids in cell adhesion, an important property for the organisation of multicellular organisms.

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Table 1 Adhesiveness of synthetic and natural lipids to monolayers of HeLa cells

Lipids	Adhesiveness of lipid particles (fluorescence intensity*)	Adhesiveness of erythrocytes (haemoglobin†)
A Ceramide-Glc	18	0.03
B Ceramide-Gal	25	0.04
C Ceramide-Gal-SO_4^-	91	0.25
D Ceramide-Fuc	20	0.01
E Ceramide-Glc-Gal	40	0.12
F Ceramide-Gal-Gal-Gal	71	0.23
G Ceramide-Gal-Gal-Gal-Gal	92	0.23
H Ceramide-Glc-Glc-Glc-Glc	49	0.12
I Ceramide-Glc-Gal-Gal-GalNAc	31	0.13
J Ceramide-Glc-Gal-GlcNAc-Gal	90	0.27
K Ceramide-Glc-(NeuNAc)Gal-GalNAc-Gal-NeuNAc	37	0.18
L Ceramide-Glc-Gal-GalNAc-Gal	100	0.35
M Sphingomyelin	23	0.08
N Phosphatidyl cholin	20	0.07
O Phosphatidyl ethanolamine	21	0.07
P Phosphatidyl inositol	24	0.08

A, Isolated from a Gaucher spleen[23]. *B*, Isolated from human brain[24]. *C*, Isolated from human brain[24]. *D–H*, Chemically synthesised by coupling ceramide with fucose, lactose, trigalactoside, tetragalactoside and tetraglucoside, respectively. Ceramide was obtained from brain cerebroside by the method of Carter *et al.*[25]. L-Fucose and lactose were from Serva. Trigalactoside and tetragalactoside were obtained from gum arabic (Serva) by partial hydrolysis with 0.2 M HCl at 100° C for 2 h and isolation from a column of active charcoal[26]. Tetraglucose was obtained from potato starch by the same procedure. Coupling of ceramide with oligosaccharides was performed via acetobromo derivatives of oligosaccharides with silver oxide as catalyst[27]. By this procedure the oligosaccharides were exclusively linked to the C-1 of ceramide. The yields of glycolipids were in general 3–10% of the theoretical value. The products were purified by silicic acid chromatography using increasing concentrations of methanol in chloroform (10%, 25%, 50%), methanol and finally with 10% concentrated ammonia in methanol as eluting solvents. Fractions were monitored by thin layer chromatography using chloroform–methanol–water (65–35–8) as the developing solvent. *I*, Globoside, isolated from MDBK cell as the major glycolipid. *J*, Obtained by chemical coupling of ceramide with the corresponding tetrasaccharide isolated from human milk[28], using the same procedure as described for *D–H*. *K*, Ganglioside isolated from human brain[29]. *L*, Ceramide-tetraoside. Obtained from ganglioside (G_{D1a}) by repeated hydrolysis with 0.02 M HCl at 80° C. The liberated *N*-acetylneuraminic acid and the HCl were removed by dialysis. Lipid particles containing these glycolipids or phospholipids were prepared essentially as described elsewhere[15], except that ratio of the components of the particles were modified to glycolipid or phospholipid–cholesterol–triolein–dansylsphingosine 1.2.0 1:0.01. The technique of binding assay of lipid particles is described in the legend to Fig. 1. The extent of adhesiveness was measured by the fluorescence intensity of adhered lipid particles. To measure fluorescence, the washed monolayers were left with 3 ml of ethanol for 10 min at room temperature and the ethanol extracts were measured in a Perkin-Elmer fluorescence spectrophotometer. In the case of adhesion studies using erythrocytes, lipids were incorporated into erythrocyte membranes as described in Fig. 2 at 5 μg lipid per incubation mixture. The treated erythrocytes were then incubated with monolayers of HeLa cells as for Fig. 2. Adhesion was measured by the haemoglobin content of adhered erythrocytes. For this purpose, monolayers were scraped off the plates in 2 ml of phosphate-buffered saline (PBS). Haemoglobin was then liberated by repeated (3×) freezing and thawing. After centrifugation at 2,000*g* for 10 min the absorption of the clear supernatant was measured at 540 nm.

* Excitation at 340 nm and emission at 500 nm. All values were relative to that of compound *L* set at 100.

† Extinction at 540 nm.

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5,6-Dihydroxyindole is a melanin precursor showing potent cytotoxicity

WE have previously suggested that factors which regulate the proliferation and pigmentation of Cloudman S-91 mouse melanoma cells share common biochemical pathways[1,2]. Our conclusion was based on the following observations: (1) most mutant cells selected for their ability to grow in culture medium containing melanocyte-stimulating hormone (MSH) show an altered ability to produce pigment[1], and (2) tyrosinase is activated in response to elevated levels of cyclic AMP in a reaction apparently mediated by a cyclic AMP-dependent protein kinase[3]. This same protein kinase has been implicated as a positive regulator in the proliferation of Cloudman cells[4]. In addition, we found that some precursors of melanin are cytotoxic and that cells actively producing melanin run the risk of self-destruction through a build-up of these precursors. We have previously shown that pigmented cells are killed as a result of exposure to excess tyrosine or dihydroxyphenylalanine (dopa) in the culture medium[2,5]. It has since been demonstrated that actively melanising cells release a cytotoxic agent into their culture medium[6]. Here we present further evidence for the cytotoxicity of melanin precursors and show that 5,6-dihydroxyindole is one of the toxic compounds in the melanin biosynthetic pathway.

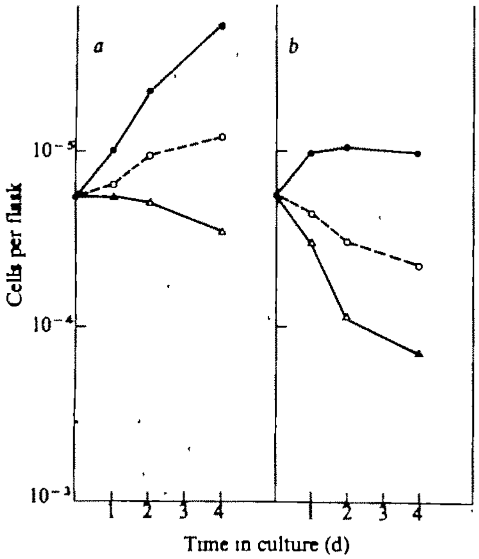


Fig. 1 Effects of dopa and MSH in combination with theophylline on cellular proliferation. Cells (2×10^4) were inoculated into Corning 25-cm^2 tissue culture flasks. The next day medium was removed and replaced with fresh medium containing no additions (●), 1×10^{-4} M dopa; (○), or 2.5×10^{-4} M dopa (△). *a*, Medium containing no MSH or theophylline. *b*, Medium containing MSH (2×10^{-7} M) and theophylline (5×10^{-4} M). At the times indicated cultures were collected in triplicate and counted in a Coulter counter. Variation within triplicates was less than ±20%. These experiments were repeated three times with similar results.

From various clinical and experimental observations it was concluded that during melanisation, pigment cells produce substances which are potentially autotoxic, and it was predicted that a strong stimulus for the synthesis of melanin would raise the intracellular concentration of tyrosine, dopa and their derivatives and increase the likelihood of self-destruction of the melanocytes[7-9]. This prediction was supported by results of studies with cultured mouse and hamster melanoma cells. Tyrosine, dopa and tryptophan were toxic to melanotic cells but not to amelanotic mouse L-cell fibroblasts, although dopa caused a slight retardation of the L-cell growth rate (Table 1). Other naturally occurring amino acids had little or no effect on either cell type. Cells with high tyrosinase activity that resulted from exposure to MSH were much more susceptible to the toxicity of melanin precursors than were cells not exposed to the hormone (Fig. 1 *a*, *b*). Treatment of cells with phenylthiourea, a potent and selective inhibitor of tyrosinase, provided complete protection from the toxicity of the melanin precursors[2]. Together, these results indicate that one or more products of the tyrosine–tyrosinase reaction are cytotoxic and that for cultured Cloudman S-91 melanoma cells, the earlier predictions[7] mentioned above are correct.

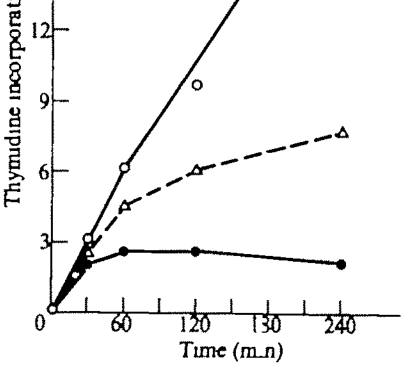
Table 1 Effects of various amino acids on the viability of melanoma cells

Additions to culture medium	Cell count (% of controls) after 6 d in culture	
	Melanoma cells	L cells
None	100	100
Ala, Arg, Asp, AspNH$_2$, Cys, Glu, GluNH$_2$, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Val	100	100
Dopa	<1	65
Tryptophan	1	100
Tyrosine	12	100

Mouse L cells or Cloudman S91 melanoma cells (2×10^4) were inoculated into 25-cm^2 Falcon tissue culture flasks in 4 ml F10 culture medium. The next day the cells were fed media supplemented individually with each amino acid indicated. All concentrations of amino acids were 5×10^{-3} M except dopa (1×10^{-4} M). Media were changed every 2 d and on the 6th day the cells were collected and counted in a Coulter counter. Control cultures of L cells had a generation time of 22 h, and control cultures of melanoma cells had a generation time of 43 h. These experiments were repeated twice, with similar results each time.

We next attempted to determine the nature of the compounds responsible for the cytotoxicity. Cultured cells were exposed for

Fig. 2 Effects of dopa and 5,6-dihydroxyindole on the rate of thymidine incorporation into acid-precipitable material. Cells were inoculated into Linbro wells as described in Table 2. At the times indicated the amount of ^{3}H-thymidine incorporation into acid-precipitable material was determined in quadruplicate wells. Variation was less than ±15%. The experiments were repeated twice, with similar results each time. ○ Controls; △, dopa (1×10^{-4} M); ●, 5,6-dihydroxyindole (1×10^{-4} M).



a few hours to various agents and their effects on the incorporation of ^{3}H-thymidine into acid-precipitable material were determined. In the first experiments cells were exposed to dopa (4×10^{-4} M) in the presence or absence of ascorbic acid (1×10^{-3} M) as a reducing agent[10]. After 16 h, cells in medium containing dopa alone were highly melanised and many were lysed. The culture medium was dark, and thymidine incorporation into acid-precipitable material was reduced to background levels (Table 2). In contrast, cells exposed to both dopa and ascorbic acid had the same morphology as control cells and the culture medium was clear. Thymidine incorporation was depressed in cells exposed to both dopa and ascorbic acid but it was significantly above background levels. Cells exposed to ascorbic acid alone appeared normal and the culture medium was clear, even though ascorbic acid reduced thymidine incorporation by about 33%. In a parallel experiment, cells were exposed to synthetic melanin (0.1 mg ml^{-1}) for 16 h. Melanin had no effect on cellular morphology and thymidine incorporation by cultures exposed to melanin was the same as that of control cultures (Table 2). Although these data do not rule out the possibility that dopa exhibited some inherent toxicity, our simplest interpretation of the results presented in Table 2 was that ascorbic acid, as long as it was present in the medium, reduced dopa quinone back to dopa and that the toxic effects were not due to dopa *per se* but rather to a compound(s) formed after dopa oxidation. As melanin itself was not toxic it seemed likely that the results were due to substances appearing after the oxidation of dopa but before the formation of melanin.

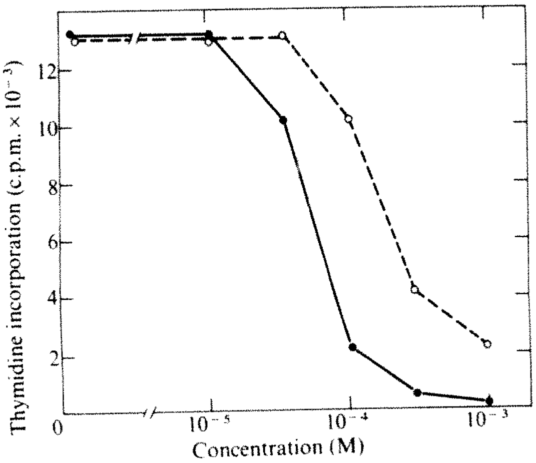
Table 2 Effects of dopa, ascorbic acid and melanin on thymidine incorporation by Cloudman S-91 cells

Additions to culture medium	^{3}H-Thymidine incorporation (c.p.m. per 10^5 cells per h)
None	1,694
Dopa	83
Dopa + ascorbic acid	605
Ascorbic acid	1,209
Melanin	1,630

Cells (10^5) were inoculated into Linbro multi-dish Disposo trays (2-cm^2 wells). After the cells had attached to the surface, medium was removed and fresh medium was added containing methyl-^{3}H-thymidine (0.3 Ci m mol^{-1}), dopa (4×10^{-4} M), ascorbic acid (1×10^{-3} M) and melanin (0.1 mg ml^{-1}, Sigma) as indicated. After 16 h the amount of ^{3}H-thymidine incorporated into acid-precipitable material was determined by the following procedure. Cells were removed from the wells with EDTA (1 mM) in Ca-Mg free Tyrode salt solution, centrifuged (2,000*g*, 5 min), resuspended in NaCl (156 mM) and precipitated with an equal volume of ice-cold trichloroacetic acid (10%, containing 1 mg ml^{-1} non-radioactive thymidine). After 15 min at 4°C the acid precipitates were filtered onto 0.45-μm Millipore filters and washed with 30 ml ice-cold trichloroacetic acid (5%, containing 1 mg ml^{-1} non-radioactive thymidine). The filters were dried in air and counted in a liquid scintillation counter. Counts represent the average from 4 separate cultures and the variation was less than ± 15%. These experiments were repeated three times, with similar results each time.

To test this interpretation, we examined the effects of 5,6-dihydroxyindole on cellular morphology and thymidine incorporation. This compound was chosen because of its ease of synthesis[11] and because it is only one step removed from melanin in the synthetic pathway. Cells were exposed to dopa (2×10^{-4} M) or 5,6-dihydroxyindole (2×10^{-4} M) simultaneously with ^{3}H-thymidine, and we found that the latter was considerably more effective than dopa in reducing the rate of thymidine incorporation into acid-precipitable material (Fig. 2). After 4 h, cells exposed to 5,6-dihydroxyindole were lysed and the culture medium was dark. In contrast, cells exposed to dopa for 4 h were not different from controls. Mouse L fibroblasts were also killed by 5,6-dihydroxyindole, indicating that this compound, in contrast to tyrosine, dopa and tryptophan, is not selectively toxic to pigment cells. A dose–response curve was constructed by comparing the effects of various concentrations of dopa and 5,6-dihydroxyindole on ^{3}H-thymidine incorporation (Fig. 3). About five times more dopa than 5,6-dihydroxyindole was required to achieve a 50% reduction in the incorporation of thymidine. These results suggest that 5,6-dihydroxyindole is at least one of the melanin precursors responsible for the cytotoxic effects observed after the oxidation of tyrosine and dopa by tyrosinase.

Fig. 3 Effects of various concentrations of dopa and 5,6-hydroxyindole on thymidine incorporation into acid-precipitable material. Cells were inoculated into Linbro wells as described in Table 2. Dopa (○) and 5,6-dihydroxyindole (●) were added at the concentrations indicated. After 4 h the amount of ^{3}H-thymidine incorporation into acid-precipitable material was determined in quadruplicate wells. Variation was less than ± 15%. The experiments were repeated three times, with similar results each time.



We have demonstrated that the generation of toxic products during melanogenesis is a characteristic unique to pigment cells, because tyrosinase is a gene product of these cells. Thus, tyrosine and dopa are specifically toxic to cells possessing tyrosinase activity whereas 5,6-dihydroxyindole shows a wider range of toxicity and is not specific to pigment cells. We hoped to develop these findings into a rationale for the chemotherapy of human melanoma. It is possible, however, that normal melanocytes have a protective mechanism against the toxicity of melanin precursors, as highly melanised but healthy cells are present throughout the animal kingdom and can also survive in culture for a considerable time[12]. Our present goals therefore, are to identify any protective mechanism(s) involved and to understand more about the factors regulating tyrosinase activity during melanogenesis.

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Do radiation-induced thioguanine-resistant mutants of cultured mammalian cells arise by HGPRT gene mutation or X-chromosome rearrangement?

RESISTANCE to the cytotoxic purine analogue 6-thioguanine (TG) in cultured mammalian cells is known to be associated with deficiency in the X-chromosome-linked purine-salvage enzyme, hypoxanthine-guanine phosphoribosyl transferase (HGPRT, EC 2.4.2.8). The induction of mutations to TG resistance in cultured mammalian cells has been used to quantify the mutagenic effects of various physical and chemical agents[1-3] and it has been argued that such mutations arise primarily as a result of true gene mutations[4]. However, because ionising radiation induces TG-resistant mutants with very low HGPRT activity[5] and fails to induce the ouabain-resistance phenotype in mammalian cells[6,7], it has been suggested that ionising radiation leads to gross genetic damage rather than to point mutations in structural genes[2,7]. Here we present evidence that gross structural changes involving the X chromosome are the genetic basis of a significant proportion of radiation-induced mutation to TG resistance in cultured human fibroblasts.

If radiation-induced mutation to TG resistance (HGPRT-deficiency) arises in cultured human fibroblasts as a consequence of gross changes to the organisation of DNA, a proportion of the more extreme changes may be visible as aberrations in the X-chromosome region containing the HGPRT locus. Gene mapping experiments have assigned the HGPRT locus in man to the terminal region of the long (q) arm of the X chromosome[8] and using chromosome banding techniques we examined the complete karyotypes of radiation-induced TG-resistant mutants of cultured human fibroblasts so as to determine whether X-chromosome changes correlated with HGPRT deficiency.

Aberrations (exchanges and deletions) consistent with the mapped position of the HGPRT locus were apparent in the X chromosome of up to 40% TG-resistant mutants induced by various ionising radiations. Figure 1*a* is a summary of the pattern disruption points (PDPs) determined for 23 mutants induced by 250-kV X rays, carbon ultrasoft X rays, helium ions of 90 keV μm^{-1}, α particles from plutonium ($\sim$140 keV μm^{-1}) and nitrogen ions of 470 keV μm^{-1}. In the case of exchange aberrations the independence of the mutants described is clear, as all the exchanges were different. The majority of mutants with X-chromosome deletions were detected in a single experiment using nitrogen ion irradiations, and it is possible that two examples of del (X) (q26) were repeats of a single clone. With a single exception (an Xp exchange probably not associated with the mutant phenotype) all PDPs occurred in the q arm of the chromosome; from the clustering of exchange PDPs in the terminal region it may be concluded (bearing in mind the inherent 3-band uncertainty of PDP allocation[9]) that the locus determining the mutant phenotype is situated distal to Xq 22. This observation agrees with most other data on the chromosomal position of the HGPRT locus in man.

A direct association between induced TG resistance and X-chromosomal aberrations would require that such aberrations in non-mutant cells surviving the radiation should be rare. In agreement with this, we failed to detect aberrations in the X chromosomes of 25 non-mutant viable clones surviving the same dose of α particles as that used to induce the mutants. On the other hand, because the wild-type culture was of female origin the aberrations detected could be in the inactive X and unassociated with the mutant phenotype. We have not rigorously excluded this possibility, but in the case of two X-chromosome exchanges (mutants III and IV of Fig. 1*b*) the aberrant X chromosome completed localised DNA synthesis before the normal X (Fig. 2). Late replication is normally associated with the inactive X and hence we conclude that in these mutants the aberrant X was the active species and was probably responsible for the mutant phenotype. Finally, it should be pointed out that techniques of mutant selection do not exclude the possibility that some mutants with X aberrations might have arisen spontaneously during post-irradiation culture. However, examination of 20 spontaneous mutant

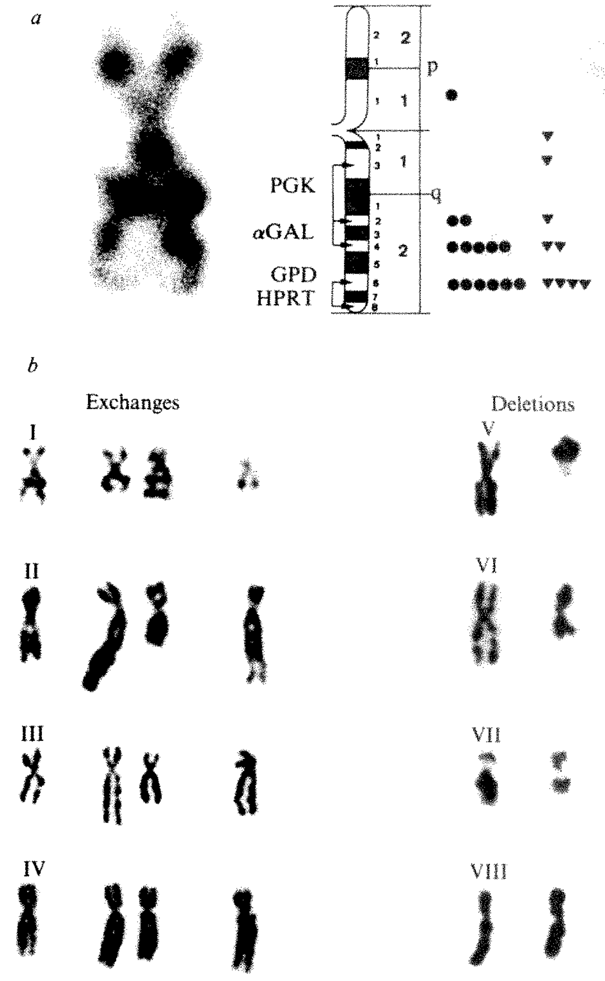

Fig. 1 *a*, Normal and schematic G-banded human X chromosome showing pattern disruption points detected in aberrant X chromosomes of a variety of thioguanine-resistant mutants of cultured fibroblasts induced by ionising radiations. ●, Exchange points; ▼, deletion points. Approximate positions for gene loci of PGK (phosphoglycerate kinase), α GAL (α galactosidase), GPD (glucose-6-phosphate dehydrogenase) and HGPRT (hypoxanthine-guanine phosphoribosyl transferase) are indicated. *b*, Examples of normal and derivative chromosomes from eight representative mutants (all 46 XX) induced by ionising radiations. Exchanges (I–IV): normal X chromosomes to the left, normal autosome to the right, derivative chromosomes central. I, t(X: 17) (q 24; q 25), mutant induced by carbon ultrasoft X rays; II, t(X; 5) (q 26; q 13), mutant induced by 250-kV X rays; III, t(X; 5) (q 24; q 13) and IV, t(X; 4) (q 26; q 31), mutants induced by α particles from plutonium. Deletions (V–VIII): normal X chromosomes to the left, deleted X to the right. V, del (x) (q 11); VI, del (X) (q 26), mutants induced by nitrogen ions of LET 470 keV μm^{-1}; VII, del (X) (q 24), mutant induced by α particles from plutonium. (Nomenclature according to Paris Conference[14]). Mutants were selected as being resistant to 3 μg ml^{-1} TG 7 d after radiation doses giving surviving fractions of 0.2 (refs [illegible], 15). All mutants were karyotyped using the ASG method[16]. The majority (including I, II and V–VIII) were karyotyped immediately after dissociation of selected mutant clones. This was a destructive technique and did not allow further study of these mutants. Six mutants induced independently by α particles were isolated and cultured before karyotyping; of these, three (III, IV and one other) showed unequivocal X-chromosome aberrations.

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karyotypes failed to reveal any structural abnormalities in the X chromosomes, so such events are too rare to account for the numbers we have observed in irradiated cultures.

The conclusion that structural changes to the X chromosome result in the loss of HGPRT raises the question of whether the function of other Xq genes are also affected. As a preliminary test of this we examined the activity of glucose-6-phosphate dehydrogenase (GPD, EC1.1.1.49) using a semi-quantitative histochemical staining technique[10]. As judged by staining intensity, the variations in GPD activity among α-particle-induced TG-resistant clones was considerably greater than that observed among non-mutant surviving clones, with up to 5% of mutant clones showing very low activity and a similar number showing super-activity. In electrophoretic enzyme assays (Fig. 3) two karyotypically defined Xq exchange mutants were completely lacking in HGPRT but were not obviously deficient or super-active in GPD. However, because almost all mutant clones showing atypical GPD activity in histochemical assays showed very poor growth it has not yet been possible to carry out truly representative karyotypic and electrophoretic analyses, and hence the question of multiple gene effects in radiation-induced mutants remains unanswered.

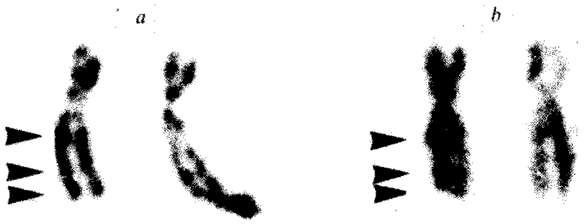

Fig. 2 Late replication of normal X-chromosome regions in two ♀ TG-resistant mutants with X-chromosome aberrations. *a*, Mutant III of Fig. 1*b*; *b*, mutant IV of Fig. 1*b*. For each chromosome pair the normal X is to the left. Late replicating regions of chromosomes were detected using the 5-bromodeoxyuridine (BUdR)–thymidine–33258 Hoechst method of Grzeschnik, Kim and Johannsman[17] with the modification that Jenner stain was used in place of Giesma. With this method late replication of a chromosome region is characterised by dark staining (indicated by arrows); early replicating regions (which incorporate BUdR into DNA) show lighter staining because the reaction of 33258 Hoechst with BUdR-substituted DNA causes that DNA to bind less Giesma or Jenner stain. The lighter staining of the q bands of the aberrant X chromosomes indicate that they were replicated early in the cell cycle.

From the karyotypic analysis presented in Fig. 1*b* it is possible to explain the TG-resistance phenotypes of radiation-induced mutants without invoking structural gene mutations. In the case of the deletion mutants (Fig. 1*b*, V–VIII), TG resistance probably arises by complete loss of the HGPRT gene from the karyotype in the acentric fragment which resulted from an incomplete radiation-induced structural aberration. For the exchange mutants (Fig. 1*b*, I–IV), loss of HGPRT could result from gene inactivation as a consequence of position effect[12] or, alternatively, the apparently simple reciprocal exchange configuration observed in mutants many generations after induction may be the stable manifestation of a more complex initial exchange event resulting in the loss of the HGPRT gene in a small interstitial chromosome deletion. The relatively poor resolution obtained with karyotypic analysis means that many small Xq changes will be undetected. It is therefore possible that the majority of radiation-induced TG-resistant mutants of cultured human fibroblasts arise by a process involving changes in chromosome structure rather than by true gene mutation. These conclusions on the nature of radiation-induced mutations in cultured mammalian somatic cells agree generally with *in vivo* observations on radiation-induced mutations in mouse germinal cells, many of which show phenotypic characteristics consistent with chromosomal changes[13].

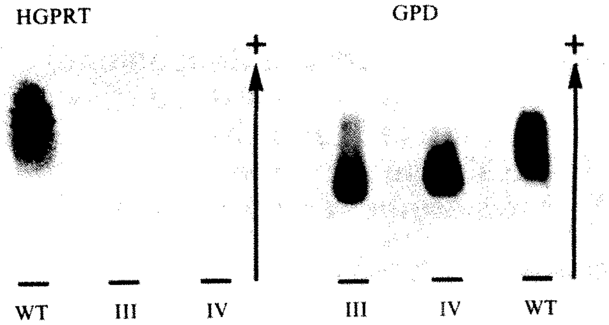

Fig. 3 Cellulose acetate gel electrophoresis of hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and glucose-6-phosphate dehydrogenase (GPD) in extracts of wild-type and TG-resistant mutants of cultured human fibroblasts. WT, wild type; III and IV refer to the exchange mutants of Fig. 1*b*. Cells (2×10^6) washed twice with isotonic salts solution were lysed for 15 min at 0 °C in 0·1 ml Tris–HCl buffer (50 mM), *p*H 8.0, 0.1% Triton. Cell debris was sedimented by centrifugation at 1,000 r.p.m. for 10 min at 0 °C; 5-μl samples of the supernatant were used for each enzyme determination. Cellulose acetate gel plates (60 × 75 mm), applicators and electrophoresis chambers were all Zip Zone (Helena). The methods were as follows. HGPRT: electrophoretic buffer, Tris–citrate (15 mM), *p*H 7.6; separation at 200 V for 45 min with ice cooling. Reaction mixture: Tris–HCl buffer (50 mM), *p*H 7.4, 2.0 ml; ^{14}C-hypoxanthine (25 μCi ml^{-1}), 0.3 ml; $MgCl_2$ (0.25 M), 0.4 ml; phosphoribosyl pyrophosphate (0.01 M), 0.3 ml. A DEAE paper overlay method with subsequent autoradiography[18] was used to determine the location of the ^{14}C reaction product (inosine mnophosphate). GPD: electrophoretic buffer, Tris-glycine, *p*H 8.5 (3.0 g Tris, 14.4 g glycine w/w per l); separation at 200 V for 40 min with ice cooling and 2 μg NADP added to the sample to stabilise GPD. Reaction mixture: Tris–HCl buffer (50 mM), *p*H 8.0, 1.0 ml; $MgCl_2$ (0.25 M), 0.1 ml; NADP (1 mg ml^{-1}), 1.0 ml; phenazine methosulphate (2.5 mg ml^{-1}), 0.1 ml; monotetrazolium (10 mg ml^{-1}), 0.1 ml; glucose-6-phosphate (0.5 M), 0.2 ml. The staining procedure was to mix the reaction solution with 2 ml molten aqueous agarose (1.4%) at 60 °C and then pipette over the gel plate. The tetrazolium reaction was allowed to proceed for 20 min at 37 °C in darkness, after which the agar was washed from the plate and the plate subsequently washed and dried.

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Competition of normal β chains and sickle haemoglobin β chains for α chains as a post-translational control mechanism

ADULT human haemoglobin (HbA) is a tetrameric protein with two α and two β polypeptide chains. Theoretically, individuals heterozygous for a β-chain mutation should have equal amounts of the normal HbA ($\alpha_2^A\beta_2^A$) and an abnormal variant ($\alpha_2^A\beta_2^X$) in their red cells. However, these people usually have different amounts of the two haemoglobins. For example, the red cells of sickle cell trait individuals usually contain about 60% HbA and 40% HbS[1-5]. Those who also have an α-thalassaemia gene(s), which decreases production of α chains, have an even lower proportion of HbS[6-12]. Each of these two haemoglobin polymers has an α-chain type common to both proteins as well as a specific β^A or β^S chain type. The individual α, β^A, and β^S chain types can be isolated *in vitro* in a functional form so that the haemoglobin tetramers can be reconstituted from appropriate mixtures of α and β chains[13]. Here we report that more HbA than HbS was formed when a mixture of equal amounts of β^A and β^S chains was incubated with limiting concentrations of α chains. These results suggest that a similar form of post-translational control of haemoglobin assembly may exist in some erythroid cells *in vivo*. The accomplishment of these experiments and the interpretation of the results were greatly facilitated by the use of radiolabelled haemoglobin chains of high specific activity.

Red blood cells were obtained from a normal human adult and from an individual with sickle cell anaemia. The sickle blood cells were incubated with ^{14}C-leucine in a mixture supporting protein synthesis[14], to provide a source of ^{14}C-labelled β^S chains. Haemolysates of both normal and sickle cells were treated with *p*-chloromercuribenzoate, which reacts covalently with protein sulphhydryl groups, to dissociate the haemoglobins into the haem-containing subunit α and β chains[13]. The β^A chains were isolated from the normal haemolysate by the procedure of Geraci *et al.*[15], and the ^{14}C-labelled β^S chains were isolated from the sickle haemolysate by the modification of McDonald and Noble[16] using a column of DEAE–Sephadex A50. α Chains were prepared with a ^{3}H label so that the relative amounts of HbA and HbS formed in the reaction with β chains could be measured quantitatively. To provide α chains of high specific radioactivity, blood reticulocytes with unusually high haemoglobin synthetic activity were obtained from an individual with Hb Sabine, a β-chain variant, and were incubated with ^{3}H-leucine[17]. The Hb Sabine was isolated from the haemolysate by electrophoresis in columns of polyacrylamide gel, and normal, ^{3}H-labelled α chains were prepared from the abnormal haemoglobin by the method of Geraci *et al.*[15].

To form haemoglobin tetramers in physiological conditions, it was necessary for the isolated α and β chains to be entirely free of mercuribenzoate. This was accomplished by treatment of the mercurated chains with mercaptoethanol to regenerate the sulphhydryl groups, as described elsewhere[15,16]. We found less than 0.01 mol of Hg for each mol of chain when our preparations were analysed for the presence of residual Hg by atomic absorption spectrophotometry. All isolated chains were dialysed against 0.01 M potassium phosphate, *p*H 7.0, and stored at 4 °C under an atmosphere of carbon monoxide.

Various amounts of ^{3}H-labelled α chains were added to replicate mixtures containing excess, but equal, amounts of β^A and ^{14}C-labelled β^S chains. The reaction mixtures were incubated at 4 °C for 60 min, sufficient time for the isolated α and β chains to react completely and form Hbs A and S. The haemoglobin products and excess chain reactants were separated by electrophoresis of a sample of each reaction mixture on a strip of cellulose acetate. The distribution of ^{3}H radioactivity among the various protein components was determined by analysis of sections of the strip in a liquid scintillation system[14].

Figure 1 shows the results from a reaction in which the total concentration of β chains was eight times greater than that of α chains. About 40% of the total ^{3}H radioactivity migrated as HbS whereas 60% migrated as HbA. These data indicate that more α chains reacted with β^A chains to form HbA than with β^S chains to form HbS. In a control experiment, unreacted free α chains migrated in this electrophoretic system near the line of application at a position well separated from the haemoglobin tetramers (data not shown). The use of ^{3}H-labelled α chains allowed the precise detection of small amounts of haemoglobin products in the presence of excess β chains. Most of the ^{14}C-labelled β^S chains migrated slightly anodal to HbA in a position characteristic of unreacted β^S chains. As expected, a small fraction (about 10%) of the ^{14}C-labelled β^S chains migrated as HbS (Fig. 1). The high (>90%) recovery of β^S chain ^{14}C radioactivity in these two components eliminates selective instability or loss of β^S chains as explanations for the formation of less HbS than HbA.

Figure 2 shows the results from several reactions in which various concentrations of α and β chains were used. In each experiment, the initial amount of β^A chains was equal to that of ^{14}C-labelled β^S chains. The amount of HbS formed was less than that of HbA in all experiments in which α chains were present in limiting amounts (α/total β ratio<1). In these experiments, essentially all the ^{3}H-labelled α chains reacted to form HbA or HbS. Conversely, when α chains were present in an amount equal to or greater than that of total β chains, the amount of HbS formed was similar to that of HbA. More than 90% of the available ^{3}H-labelled α chains reacted to form Hbs S and A when the initial concentration of α chains was identical to that of total β chains. As expected, about half (47%) the ^{3}H-labelled α chains reacted to form the two haemoglobins in equal amounts when the initial concentration of α chains was twice that of total β chains. These results indicate that essentially all the β^A and β^S chains were able to combine with the complementary α chains in the reaction mixtures. Thus, our observation of a decreased production of HbS with limiting concentrations of α chains cannot be due merely to the existence of a fraction of β^S chains unable to react with the α chains.

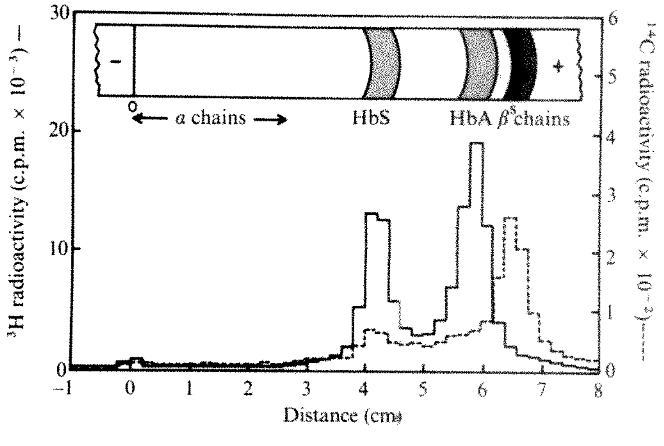

Fig. 1 Pattern of protein radioactivity after electrophoresis of a reaction mixture of α, β^A, and β^S chains. The mixture had 62 µg of ^{3}H-labelled α chains and 246 µg each of β^A and ^{14}C-labelled β^S chains in a total volume of 0.040 ml of 0.01 M potassium phosphate, *p*H 7.0. After incubation for 60 min at 4 °C, a sample of 5 µl of the reaction mixture was applied to a cellulose acetate strip. The electrophoresis was done, and the strip was cut into sections for radioactivity analysis, as described previously[14]. The positions of the stained bands of product haemoglobins and residual reactant chains are indicated at the top of the figure, and the line of sample application is denoted by zero. The β^A chains migrated off the anodal end of the strip during the electrophoresis. Almost 100% of the ^{3}H- and 91% of the ^{14}C-radioactivities applied to the strip were recovered in the sections.

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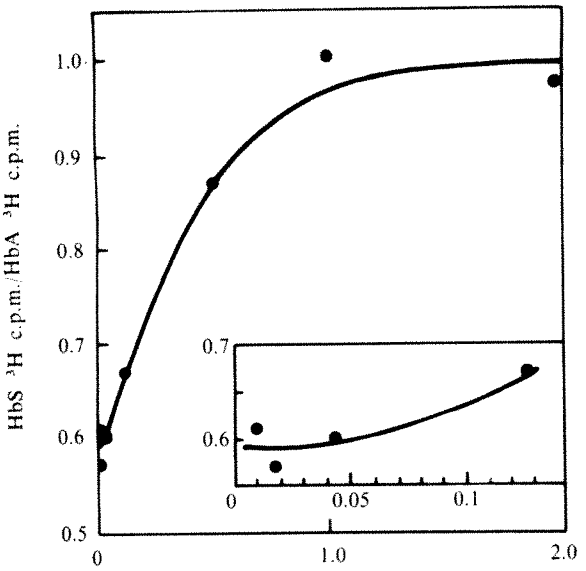

Fig. 2 Comparison of product haemoglobin radioactivities with the relative, initial concentrations of reactant chains for several experiments similar to that described in the legend to Fig. 1. The HbS/HbA ^{3}H-radioactivity ratio represents the relative amounts of ^{3}H radioactivity detected in the two haemoglobins after electrophoresis. The $\alpha/(\beta^A+\beta^S)$ chain ratio represents the relative initial amounts of α and total β chains. The inset shows the results from four experiments in greater detail. There were initially 246 µg each of β^A and β^S chains in these four reaction mixtures, with $\alpha/(\beta^A+\beta^S)$ chain ratios <0.2. The three reaction mixtures with $\alpha/(\beta^A+\beta^S)$ chain ratios of 0.5, 1.0 and 2.0 had initially 49, 25 and 12.5 µg, respectively, of each type of β chain in a total volume of 0.040 ml.

The ratio of HbS to HbA formed in these reactions fell with decreasing initial relative α-chain concentration (Fig. 2). However, when the relative α-chain concentration was sufficiently low (α/ total β ratio <0.05), the ratio of formation of HbS to HbA remained constant at about 0.6 (Fig. 2, inset). These HbS/HbA ratios represent amounts detected after complete reaction of the reagent chains during the 60-min incubation period. Although these were equilibrium measurements, the limiting value of about 0.6 for this ratio probably reflects the rates at which individual β^S and β^A chains reacted with α chains. When the α-chain concentration was increased, the resulting HbS/HbA ratio went from 0.6 to unity, as enough α chains were now available to combine with both β^S and β^A chains.

The β-chain monomer is probably the molecular species which reacts with α chains. As implied above, the difference in amounts of HbS and HbA formed in these studies probably occurs because of a difference in the relative affinities of β^S and β^A chain monomers for α chains. However, the following alternative explanation for our results remains to be eliminated. β Chains prepared by methods similar to those used in the present work exist almost exclusively in the tetrameric form, even at concentrations 50-fold lower than those used in our experiments[18-23]. The difference in amounts of HbS and HbA formed could conceivably be caused by a difference in the initial concentrations of β^S and β^A monomers resulting from unequal rates of dissociation of β_4^S and β_4^A tetramers rather than by a difference in affinities of β-chain monomers for α chains.

A similar difference in affinities of β^S and β^A chains for α chains was reported by Abraham and Huisman[24] and more recently by Lee *et al.*[25]. However, in their experiments, mercuribenzoate, added to cause tetramer dissociation during chain preparations, was still covalently bound to the subunits. Following mixture of the mercurated α and β chains, mercaptoethanol was added to aid the reconstitution of the haemoglobin tetramers by removal of the mercurial reagent. The direct addition of mercaptoethanol may result in the denaturation of a significant fraction of the chains. In our work, these denatured chains were removed during the preparative procedures. Moreover, we have found that, when partially mercurated chains are present in the reaction mixture, reconstitution of native haemoglobin is less efficient, and the tetramers formed migrate in a heterogeneous manner during electrophoresis. It is conceivable that the presence of the rather bulky mercury groups induces spurious recombination of α and β chains and influences the relative amounts of HbS and HbA observed. In the present work, the complete removal of the mercurial reagent before reaction of the chains ensured the reconstitution of haemoglobins in physiological conditions. Moreover, the use of ^{3}H- and ^{14}C-labelled chains enabled us to detect any chains partially damaged in preparation and therefore unable to form proper haemoglobins.

The results of our experiments suggest a type of post-translational control which may occur in the synthesis and assembly of HbA and HbS in some sickle trait erythroid precursor cells. Past studies have shown that the reticulocytes of sickle trait individuals who carry an α-thalassaemia gene have a lower rate of synthesis of α chains than of β chains[26,27]. These individuals also have an unusually low concentration of HbS (about 25–30% HbS, 70–75% HbA) in their red cells. A limiting concentration of newly synthesised α chains in the reticulocytes and other red cell precursors may contribute to the decreased concentration of HbS by a mechanism similar to that observed in these experiments. An analogous model has previously been proposed to explain the different concentrations of HbH and Hb Bart's observed in individuals with HbH disease[28,29], an α-thalassaemia disorder. Conversely, β-chain competition for α chains probably does not contribute to the difference in HbS and HbA levels in sickle trait individuals without an α-thalassaemia gene(s) and without the concomitant deficiency in reticulocyte α-chain synthesis[26,27].

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Transfer of the drug-resistance transposon Tn*5* to *Rhizobium*

TRANSPOSONS are discrete sequences of DNA that are incapable of self-replication and can insert into DNA replicons, such as chromosomes or plasmids, in the absence of the *recA* gene function[1]. Insertion can be random and where it occurs into the continuity of a gene leads to non-leaky polar mutations[1]. Some transposons carry drug resistance genes and thus insertions can be mapped as drug-resistance markers[1]. We report here that Tn*5*, a transposon coding for kanamycin resistance[2], can insert into the chromosome of *Rhizobium leguminosarum*, the bacterium responsible for nodulation and nitrogen fixation in *Pisum*, *Vicia*, *Lathyrus* and *Lens*.

P1 group R plasmids, such as RP4, are able to infect most Gram-negative bacteria[3,4] and have been valuable for establishing linkage maps for many species[5–8]. Transfer between *Escherichia coli* and *Rhizobium* usually occurs at a frequency of about 10^{-2} (ref. 5). Recently Boucher *et al.*[9] reported that when the lysogenic phage Mu was inserted into RP4 this frequency was reduced about 10^5-fold and the plasmids that were transferred were altered. Van Vliet *et al.*[10] have since shown that the same applies to transfers from *E. coli* to *Agrobacterium tumefaciens* and that those plasmids which do become established in this new host have large deletions of the Mu phage. Restriction and the functioning of at least one Mu gene (*r*23) are both involved in reducing the ability of these plasmids to become established[9,10]. Van Vliet *et al.*[10] also reported that the inability of RP4::Mu plasmids to become established in *A. tumefaciens* could be utilised to carry transposons into this species to obtain transposon mutagenesis. Tn*7* insertion and deletion mutations in *A. tumefaciens* plasmids have since been important in the genetic analysis of the role of such plasmids in tumour formation[11].

We attempted to use an RP4::Mu::Tn*7* plasmid (provided by Dr M. van Montagu, Gent) to obtain transposon mutagenesis in *R. leguminosarum*. Transfer of either the RP4 or Tn*7*-coded drug resistances from an *E. coli* donor could not be detected (frequency less than 10^{-8}) in crossing conditions in which the frequency of wild-type RP4 transfer was 10^{-2}. Although these crosses were unsatisfactory for introducing Tn*7* into *R. leguminosarum* they demonstrated that Mu insertions into RP4 could be used to prevent establishment of this plasmid in this host. Tn*5* (kanamycin) and Tn*10* (tetracycline) are probably the best studied transposons[1] and both are relatively nonspecific in their sites of integration into the *E. coli* chromosome[1]. As RP4 codes for resistance to both these antibiotics we constructed a new P1 plasmid carrying Mu (see footnote to Table 1), using a plasmid that coded only for gentamicin, spectinomycin and low-level streptomycin resistance (pPH1JI)[12]. A derivative of this plasmid carrying Mu and Tn*5*, called pJB4JI, was used as a carrier of Tn*5* into three *Rhizobium* species (Table 1). The data in Table 1 show that the ability of PJB4JI, to become established in these species was reduced relative to the parent plasmid pPH1JI. Bacteria resistant to kanamycin, kanamycin plus gentamicin and gentamicin alone were found in these crosses (Table 1). None of these was found to be able to transfer drug resistance to *E. coli* (frequency less than 10^{-8}) in conditions where pPH1JI transfer occurred at a frequency of approximately 10^{-2}.

To be useful as a generalised mutagen a transposon needs to be relatively nonspecific in its insertion. This was tested for Tn*5* by replica-plating kanamycin-resistant derivatives selected on complete medium to minimal medium and scoring for auxotrophy. For *R. leguminosarum*, *R. trifolii* and *R. phaseoli* the frequency of auxotrophy was about 0.3% (compared to a spontaneous rate of less than 10^{-8}) and the range of auxotrophs found suggests that insertion was random. Auxotrophic mutants with the following requirements have been found to date: for *R. leguminosarum*, leucine (2), adenine (5), serine (2), riboflavine (1), methionine (2), adenine or guanine (1), phenylalanine + tyrosine (1) and two with unknown requirements; for *R. trifolii*, tryptophan (2), adenine or guanine (1), leucine + isoleucine + valine (1); for *R. phaseoli*, phenylalanine (1), tryptophan (2), methionine (1), leucine (1), adenine (2), uracil (1), cysteine or methionine (2) and two with unknown requirements. These results indicate the potential for obtaining apparently random mutagenesis in *Rhizobium* species.

Because the vehicle for introducing Tn*5* into *Rhizobium* carried phage Mu, it is conceivable that the mutagenesis was

Table 1 Transfer of kanamycin (Km) and gentamicin (Ge) resistance from *Escherichia coli* to *Rhizobium* species*

Recipient	pPH1JI	Plasmid in donor† pPH1JI::Mu::Tn*5* (pJB4JI)		
	Ge	Ge	Km	Ge + Km
R. leguminosarum 726	10^{-2}	10^{-7}	10^{-6}	5×10^{-8}
R. trifolii 6709	10^{-1}	10^{-6}	5×10^{-6}	2×10^{-8}
R. phaseoli 1234	10^{-1}	10^{-6}	5×10^{-6}	1.5×10^{-8}

Crosses were performed as described by Beringer *et al.*[5] except that mating was allowed to proceed for only 4 h at 28 °C. The transfer of plasmids and resistance markers was selected on complete (TY)[5] medium supplemented with gentamicin (5 μg ml^{-1}), kanamycin (20 μg ml^{-1}), streptomycin (50 μg ml^{-1}) and rifampicin (20 μg ml^{-1}) as required. The transfer of gentamicin plus kanamycin resistance was determined by replica-plating colonies selected on a single antibiotic to medium containing the other. *R. leguminosarum* strain 726 is a rifampicin resistant derivative of our wild-type strain 300 (ref. 15). *R. trifolii* strain 6709 and *R. phaseoli* strain 1234 are streptomycin-resistant derivatives of strains 204 and 3644 from the Rothamsted Culture Collection. Plasmid pJB4JI was constructed in this laboratory by selecting Mu c^+ lysogens of an *E. coli* J53 strain (carrying the P1 group plasmid pPH1JI) after spotting Mu on to a lawn of log phase bacteria. Four lysogens were crossed to a Mu *cts* lysogen of *E. coli* (strain SB1801) with selection for gentamicin resistance carried on pPH1JI and growth at 42 °C. Gentamicin-resistant colonies growing at 42 °C were presumed to carry pPH1JI and the wild-type Mu which was required to suppress the thermoinducible Mu *cts* phage in strain SB1801. Ten clones were picked and regrown under these selective conditions before crossing with an *E. coli* J53 *nal* strain and separately with an *R. leguminosarum* recipient in patch matings as described by Johnston, Setchell and Beringer[16]. All 10 clones transferred gentamicin resistance and the ability to produce Mu phage to strain J53 *nal*; none transferred gentamicin resistance to the *R. leguminosarum* strain, though transfer of pPH1JI would have occurred. Each clone was assumed to carry Mu inserted into a part of the plasmid not carrying important transfer genes. One clone was crossed into an *E. coli* recipient (strain 1752) carrying a chromosomally inserted Tn*5*. The pPH1JI::Mu derivative of strain 1752 was crossed with a suitable *E. coli* recipient with selection for kanamycin transfer. Kanamycin-resistant transconjugants were crossed to a J53 *nal* recipient and a kanamycin and gentamicin-resistant derivative (strain 1830) was purified. Strain 1830 transferred both resistances at high frequency between *E. coli* strains and was found to be Mu immune but unable to produce viable Mu phage. This indicates that Tn*5* had inserted either into Mu or beside it, causing a deletion into Mu. The pPH1JI derivative plasmid carrying Mu and Tn*5* is called pJB4JI and the pPH1JI::Mu parent plasmid is called pJB9JI.

* Frequencies of transfer are approximate and measured per recipient plated.

† Donor was *E. coli* strain J53 *nal* carrying the plasmid shown.

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Table 2 Transduction of Tn*5*-associated auxotrophs by rhizobiophages RL38 and RL39

a Selection for the transfer of kanamycin resistance

Donor*	Frequency of kanamycin resistance (per recipient)			Proportion of kanamycin resistant recipients also receiving Tn*5*-associated auxotrophy		
	RL38	RL39	Control	RL38	RL39	Control
T94	1×10^{-5}	1×10^{-6}	1×10^{-8}	100/100	95/95	0/4
T95	8×10^{-6}	1×10^{-6}	1×10^{-8}	95/95	64/64	0/1
T133	6×10^{-6}	9×10^{-7}	$<10^{-8}$	250/250	150/150	—

b Selection for the transfer of prototrophy

Recipient	Frequency of prototrophy (per recipient)			Proportion of prototrophic recipients also kanamycin sensitive		
	RL38	RL39	Control	RL38	RL39	Control
T94	2×10^{-5}	7×10^{-7}	$<10^{-8}$	100/100	100/100	—
T95	3×10^{-5}	2×10^{-6}	5×10^{-8}	292/292	249/251	3/7
T133	2×10^{-6}	3×10^{-7}	3×10^{-8}	544/545	284/284	10/10

* T94, 95 and 133 are derivatives of the *R. leguminosarum* strain 300 carrying respectively auxotrophic requirements for adenine, leucine and arginine + uracil (*pyrA*?) resulting from the transfer of Tn*5* from *E. coli*. Phages RL38 and RL39 have recently been isolated as lytic phages for *R. leguminosarum* strain 300 (A.V.B-W., in preparation). Transduction was essentially as described by Ely and Johnson[17] for *Caulobacter crescentus*. Phages were irradiated with ultraviolet light to 0.1% survival and added to the recipient at a multiplicity of infection of about one phage per bacterium. Suitably marked strains of *R. leguminosarum* were used in these crosses so that contamination of phage preparations by viable bacteria would have been detected in the analysis of recombinants.

associated with Mu insertions into the chromosome[13]. This is unlikely for a number of reasons.

(1) Van Vliet *et al.*[10] have reported that a major factor limiting the ability of RP4::Mu plasmids to become established in *A. tumefaciens* is likely to be the formation of extensive deletions in the plasmid, which do not occur in the absence of Mu. Those few plasmids that do become established have extensive deletions of Mu. Thus the inheritance of fully functional Mu genomes would appear to be unlikely.

(2) A direct association between kanamycin resistance and induced auxotrophy was demonstrated by transduction (Table 2) and conjugation (J.E.B. unpublished results). The data for transduction are particularly convincing as relatively small pieces of DNA are transferred. They show an almost complete correlation of auxotrophy with resistance, whether selection was made for prototrophy or for transfer of kanamycin resistance (Table 2).

(3) Twelve auxotrophic mutants arising from the transfer of Tn*5* were tested for reversion to prototrophy. For seven of these mutants, reversion to prototrophy was not detected (frequency less than 10^{-8}) and for two others, occasional colonies of presumptive revertants were detected (frequency about 10^{-8}). Three strains had reversion rates of about 10^{-8}, 10^{-5} and 10^{-6}, and respectively 10%, 34% and 90% of revertants were kanamycin sensitive. The genetic basis of the reversions observed was not determined, but it can be assumed that the kanamycin-sensitive revertants were not due to suppression but to a precise excision of the insertion which had caused the mutation. Spontaneous reversion of Mu-induced mutations in *E. coli* is exceedingly rare[13], which suggests that at least the reverting mutants were not Mu-induced, unless the precise excision of Mu from the *R. leguminosarum* chromosome is more frequent than from the chromosome of *E. coli*.

This work clearly shows that a plasmid carrying Mu can be used to introduce a transposon (Tn*5*) into three *Rhizobium* species. This transfer is associated with the formation of auxotrophic mutants in these species and has been used to provide a readily selectable phenotype for at least one indigenous plasmid in *Rhizobium*[14]. We cannot be certain, at present, that Mu is not involved, but this does not detract from the value of the technique for establishing mutagenesis by an easily mapped insertion 'mutagen'. This should be of particular value in studies of the *Rhizobium*–legume symiosis as the analysis of defective mutants of *Rhizobium* depends on plant tests. Thus the mapping of symbiotically-defective mutants obtained by 'conventional' means requires that each recombinant from a cross be tested individually on a plant. However transposon-induced mutations can be mapped by mapping the position of the drug resistance carried on the transposon. Therefore transposon-induced symbiotic mutations can theoretically be mapped using the minimum of plant tests, which are expensive and time consuming.

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High frequency transfer of nodulating ability between strains and species of *Rhizobium*

THE most striking and important feature of members of the genus *Rhizobium* is their ability to nodulate and fix nitrogen in the roots of legumes. There is specificity in this interaction. Legumes belonging to one cross-inoculation group are nodulated only by certain classes of *Rhizobium* and indeed *Rhizobium* species are defined in terms of the host legumes that they can nodulate—for example, *R. leguminosarum* nodulates peas, *R. trifolii* nodulates clover and *R. phaseoli* nodulates beans of the genus *Phaseolus*. The genetic bases of the nodulation process and of host specificity are poorly understood. Many *Rhizobium* strains harbour plasmids[1–4] and genetic evidence has suggested that such plasmids might carry some of the information required for the development of the symbiosis[5,6]. We report here that the ability to nodulate peas can be transferred at high frequency from a strain of *R. leguminosarum* to a non-nodulating strain of *R. leguminosarum* and to three other species of *Rhizobium* that nodulate legumes other than peas. We infer from this that some of the genetic information required for nodulation is plasmid-linked.

In the previous paper[7] the insertion of the transposon Tn*5* (conferring kanamycin resistance) into the chromosome of strains of *Rhizobium* was described. Using the same method we attempted to insert Tn*5* into pRL1JI, a conjugative plasmid first found in an *R. leguminosarum* field isolate (strain 248). Strain 248 produces a bacteriocin and this ability can be transferred at high frequency ($\sim 10^{-2}$) to other strains of *Rhizobium*[8]. pRL1JI was transferred by conjugation into *R. leguminosarum* strain 1062 (*ura14 trp16 str69*), a derivative of strain 300 (ref. 9). One pRL1JI transconjugant, strain 2406, was used as a recipient in a cross with *Escherichia coli* strain 1830 that carries pJB4JI (ref. 7) (pPH1::Mu::Tn*5*) and selection was made for the transfer of kanamycin resistance. Resistant colonies arose at frequencies of $\sim 10^{-6}$ and 20 of them were used in patch crosses with *R. leguminosarum* and separately with *E. coli* recipients. None transferred kanamycin resistance to *E. coli* but one transferred it at very high frequencies to *R. leguminosarum*. This strain (T3) and a control that did not give kanamycin transfer in the patch crosses (T10), were each crossed with *E. coli* and *R. leguminosarum* using membrane crosses to determine frequencies of transfer. None transferred kanamycin resistance to *E. coli* (frequency $< 10^{-8}$). T3 and T10 transferred kanamycin resistance to the *R. leguminosarum* recipient at frequencies of about 10^{-2} and 10^{-8} per recipient, respectively. Recipients of kanamycin resistance from T10 may have arisen by the pick-up of Tn*5* onto a conjugative plasmid.

Strain T3 failed to produce the bacteriocin specified by pRL1JI; one possible explanation was that Tn*5* had been inserted into (or beside) the bacteriocin production gene. To test this hypothesis T3 was crossed with a strain of *R. leguminosarum* that did not produce the bacteriocin. One kanamycin-resistant transconjugant was purified and was used to propagate the transducing phage RL38 (A.V.B.-W., in preparation). The lysate was used to transduce a strain of *R. leguminosarum* that carried pRL1JI and hence produced bacteriocin. Kanamycin-resistant transductants arose at a frequency of $\sim 10^{-6}$ per recipient cell; 99.5% of them failed to produce bacteriocin, showing that kanamycin resistance was closely linked to the inability to produce bacteriocin and suggesting that Tn*5* had indeed inserted into pRL1JI and had caused tthe loss of production in strain T3.

Strain T3 was used as a donor in crosses with the strains in Table 1. In all cases kanamycin-resistant transconjugants arose at frequencies of $\sim 10^{-2}$. When each of the parental strains was plated separately on the selective media colonies occurred at frequencies of $\sim 10^{-8}$. From each cross several transconjugants as well as control clones of the recipient strain which had not acquired kanamycin resistance were used to inoculate peas.

The control clones nodulated peas rarely or not at all (Table 1). One plant inoculated with one clone of strain 6710 had a single small nodule. Such sporadic nodulation of peas by *R. trifolii* has been observed before[10]. In contrast, the transconjugants induced many nodules per plant, showing that the ability to nodulate peas could be transferred to a non-nodulating strain of *R. leguminosarum* and to other species of *Rhizobium*. Most of the *R. trifolii* and *R. phaseoli* transconjugants began to produce nodules about one week later than normal and the nodules were smaller than those found following inoculation of peas by *R. leguminosarum*. Strain 9009 is a slow-growing strain able to nodulate *Cicer*. The first appearance of nodules formed by transconjugants of this strain was delayed for a further 4–5 d and, in contrast to the other recipients, not all of them nodulated peas; two failed to do so. However all three plants inoculated by one of the *R. phaseoli* transconjugants had nodules that appeared at the normal time and were of similar size and number to those found following inoculation by *R. leguminosarum*.

The roots of a sample of the plants inoculated by transconjugants were tested for acetylene reduction as an assay for nitrogenase activity (Table 1). All the strain 6015 transconjugants were able to reduce acetylene, but the interspecific transconjugants varied in their ability to do so. All of the *R. phaseoli* transconjugants, but only a minority of those of the other two species, reduced acetylene and with a single exception the rates were low (~10% of those found following inoculation of peas by strains of *R. leguminosarum*) The exception was the *R. phaseoli* transconjugant that nodulated peas as well as the *R. leguminosarum* control; this reduced acetylene at normal rates. It is not clear why the ability to reduce acetylene varies both between the different recipient classes and between transconjugants of the same recipient. We cannot say whether the transconjugants which failed to reduce acetylene would have done so after prolonged growth of the plants, because in our conditions of growing peas the plants became distressed about five weeks after germination.

The *R. trifolii*, *R. phaseoli* and strain 9009 transconjugants were used to inoculate *Trifolium repens* (var. S100), *Phaseolus vulgaris* (var. Fino) and *Cicer arietinum* L. (var. G130) respectively, these being the normal hosts for the parents of these transconjugants. Chickpeas (*C. arietinum*) were inoculated and grown in the same way as peas; the methods used for beans and clover were essentially as have been described[9] except that clover seedlings were grown in vertically held Petri dishes (B. Rolfe, personal communication). All the interspecific transconjugants formed nodules on their original hosts but the nodules were slow to appear and were fewer and smaller than those induced by their respective parents on the same hosts. Interestingly, of the *R. phaseoli* transconjugants tested, the one that best nodulated peas was poorest on beans, inducing only a single ineffective nodule on a total of three inoculated plants.

The finding that the ability to nodulate peas was transferred from a strain of *R. leguminosarum* at high frequency is strong evidence that in this species there is a plasmid carrying genetic information required for nodulation. We cannot say whether this information is in the bacteriocinogenic plasmid pRL1JI or in another plasmid that was cotransferred at high frequency. T3

Table 1 Transfer of ability to nodulate peas and reduce acetylene in the nodules from strain T3 to other strains of *Rhizobium*

Recipient strain	Species	Markers	Control clones: No. nodulating/no. tested	Transconjugants clones: No. nodulating/no. tested	Transconjugants clones: No. reducing C_2H_2/no. tested
6015	*R. leguminosarum*	*phe1 trp12 rif +392 str37 inf*	0/9	17/17	8/8
6661	*R. trifolii*	*rif*	0/7	16/16	2/9
6710	*R. trifolii*	*rif*	1/10	17/17	1/8
1233	*R. phaseoli*	*rif*	0/6	9/9	9/9
9009	*R.* '*species*'		0/10	8/10	2/8

Strain 6015 is a non-nodulating derivative of strain 897 (ref. 14) isolated following ultraviolet treatment. In many previous studies it had never been shown to nodulate peas. Strains 6661, 6710 and 1233 are *rif* mutants of strains 12, 204 and 3644 respectively of the Rothamsted Culture Collection. Strain 9009 is strain 3889 from Rothamsted, it is a member of the cowpea miscellany. These last four strains were effective (that is, reduced acetylene) on their hosts respectively clover, clover, French bean and chickpea. Media and growth conditions for *Rhizobium*, and the pea inoculation tests have been described[15] as has the method of doing bacterial crosses[13]. The crosses were plated on minimal medium and where appropriate, rifampicin (20 μg ml^{-1}) to counterselect the donor plus kanamycin (20 μg ml^{-1}) to select transconjugants. Colonies were purified by streaking twice on the same selective medium and cultures were grown and used to inoculate two peas (var. Wisconsin Perfection). The plants were monitored for the appearance of nodules After 3–4 weeks the plants were scored for nodulation and the ability to reduce acetylene. Nodules from each of the peas nodulated by the transconjugants were excised, surface sterilised, crushed and the contents plated on complete medium. About 50 single colonies were replicated to appropriate selective media. In all cases the colonies had the appropriate markers.

was not a high-frequency donor of chromosomal markers in the cross between T3 and strain 6015 as, when selection was made for the transfer of *phe1*$^+$ or of *trp12*$^+$, progeny arose at frequencies of only $\sim 10^{-8}$. Thus nodulation ability was transferred at frequencies $\sim 10^6$-fold greater than these alleles.

Earlier studies showed that the plasmid R68.45 mediated chromosomal recombination between *R. leguminosarum*, *R. trifolii* and *R. phaseoli*[12]. The strains used were derived from the same parents as were strains 6015, 6661 and 1233 respectively. These studies have since been extended (A.W.B.J., unpublished observations). In separate crosses 16 different alleles were transferred from *R. trifolii* to *R. leguminosarum*. From each cross 20–30 recombinants were tested for their ability to nodulate clover but this was not co-inherited with any of the alleles tested. The location of the alleles chosen on the *R. leguminosarum* chromosome[13] was such that if any gene(s) for host specificity was chromosomally located and was transferred and expressed then we would have expected to have been able to map it. This negative result provided indirect evidence for plasmid involvement in host specificity.

Higashi[5] concluded that the ability to form infection threads on clover could be transferred by way of a plasmid from *R. trifolii* to *R. phaseoli*. He did not say whether the transconjugants could still nodulate their normal host. We do not know why, in our study, most interspecific transconjugants nodulated both peas and the host of the recipient, albeit with reduced efficiency in both cases. It is possible that the introduction of the plasmid did not replace the genetic information needed by the recipient strain to nodulate its normal host. Perhaps the presence in the same cell of genetic information needed to nodulate host plants of two different cross-inoculation groups causes it to be deficient in its nodulation of either. The single *R. phaseoli* transconjugant that nodulated peas well but beans barely at all may represent a case where the information needed to nodulate beans had been lost.

Physical isolation and characterisation of the plasmid(s) involved may help to clarify their role in symbiosis. Such analysis may be complex, as *R. leguminosarum* strain 300, the parent of the strains used in this study, contains three large ($MW > 10^8$) plasmids[16].

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Dynamics of protein hydration by quasi-elastic neutron scattering

THE importance of water and its properties for biological systems makes the analysis of the dynamics of protein hydration of special interest[1–3]. It has long been recognised[2] that neutron scattering can, in principle, provide the most complete description of molecular motions in hydrated biopolymers. Here we report the first results of an application of high-resolution quasi-elastic neutron scattering[4–7] to the dynamics of protein hydration. A neutron beam incident on a biological sample produces scattered radiation that consists of a coherent part (that is, one that gives rise to an interference pattern which, in its Bragg scattering, contains the structural information[8–10]) and an incoherent part that cannot give interference effects but exhibits spectral changes which carry information on dynamic processes. Quasi-elastic neutron scattering explores the low-frequency region of the spectrum characterised by energy transfers of the order of, and less than, $1\ \text{cm}^{-1}$. Because of the very large incoherent scattering cross-section of protons (80 b) relative to all other nuclei (<0.4 b) present in natural biological samples, the incoherent scattering is almost entirely due to hydrogenous groups and water molecules. By replacing some or all of the carbon-bound hydrogens by deuterium (2 b), dynamic contrast factors of up to 15 can be attained so that a wide range of contrast experiments becomes possible.

In the case of hydrated biopolymers, there are three main classes of motions that may be investigated by incoherent neutron scattering: polypeptide side-chain motions[11], intramolecular modes involving subunits and larger segments of a macromolecule, and motions associated with the water of hydration. Recent theoretical work on protein dynamics has considered thermodynamic fluctuations[12], the fluid-like nature of thermal motions on a picosecond time scale[13], segmental flexibility[14] and folding[15], and computer simulations of the solvent structure[16]. In an effort to exploit the potential of recent advances in high-resolution neutron spectroscopy[6,17,18] in studies of this kind, we have examined the dynamics of water molecules interacting with a fully deuterated globular protein at low to moderate levels of hydration.

We used two biosynthetically fully deuterated samples of the multimeric chromoprotein C-phycocyanin (MW 190,000) extracted from *Synechococcus lividus*[19–21]. Blue–green algae contain large amounts of phycocyanin and are capable of synthesising it in extreme environmental conditions. Current interest in protio- and deuterio-phycocyanin relates primarily to its photochemical properties and to the opportunities it offers as a model system for studying the inter- and intramolecular forces involved in folding and aggregation processes. One sample consisted of stacks of protein films (surface density $\approx 5\ \text{mg cm}^{-2}$) cast on thin Al foils, the other was in the form of fluffy aggregates of wisp-like particles obtained by drying from aqueous solutions. The IN10 backscattering spectrometer[17] at the Institut Laue-Langevin, Grenoble, was used to record quasi-elastic spectra at 5 or 6 scattering angles between $2\theta = 9.8°$ and 110° (corresponding to momentum transfers $k = (4\pi/\lambda_0) \sin\theta = 0.17$ and $1.64\ \text{Å}^{-1}$) within a spectral window of 0.07 or $0.11\ \text{cm}^{-1}$ centred on the incident neutron wavelength $\lambda_0 = 6.27$ Å (Fig. 1). To obtain reliable and consistent values for the small quasi-elastic broadenings, ΔE, expected for surface scattering from a deuterated biopolymer, the spectra measured for the hydrated sample were analysed, not with respect to the instrumental resolution width W_v of the quasi-elastic line (as obtained for a purely incoherent scatterer such as vanadium), but differentially with respect to the width W_r obtained for the identical, dry or very slightly hydrated sample. Three wet-minus-dry difference spectra determined for intensity-normalised lines are shown in Fig. 2. For the same reasons, this line-shape analysis was not

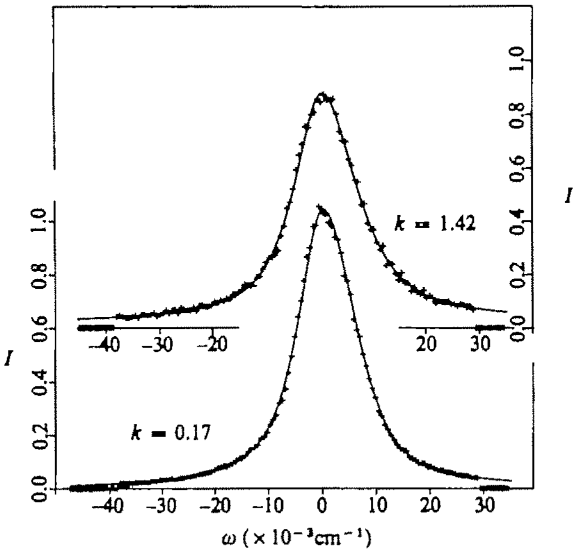

Fig. 1 Examples of quasi-elastic spectra measured (+) for phycocyanin film stacks hydrated at relative humidity $RH \leqslant 90\%$ H_2O (temperature 24 °C). I = neutron counts corrected for detector efficiency (arbitrary units); ω = energy transfer. Momentum transfer k (Å^{-1}) corresponds to scattering angles $2\theta = 9.8°$ and $90°$; resolution widths W_r (dry sample) were 0.0129 and 0.0143 cm^{-1}, respectively. Solid lines are analytical fits using asymmetric Peyre–Principi (PP) profiles[22]. The spectra shown were obtained by a backscattering technique[17] as follows. Cold neutrons from the liquid deuterium moderator (20 K) in the reactor are collimated along a 55-m guide tube. This beam consists of neutrons with a wide distribution of wavelengths in the 2–12 Å region. Reflection from a silicon monochromator crystal produces a highly monochromatic line of $\lambda_0 = 6.2708$ Å neutrons. By imparting a periodic translational motion of a few hertz to the monochromator, the neutrons selected by it are Doppler-shifted with respect to the fixed sample and continuously scan a spectral window of 0.02 Å in wavelength or 0.11 cm^{-1} in energy centred on λ_0. In a sample consisting of nuclei moving relative to each other, each elementary scattering event between neutron and nucleus is accompanied by small energy changes. Neutrons scattered by the sample are energy-analysed by 180° reflection from a fixed array of silicon crystals positioned at the scattering angle 2θ observed. The crystals forming this array reflect neutrons back on to a detector located very close to the sample.

carried out by means of conventional lorentzian fitting routines. Instead, we attempted to define a less model-dependent measure of the broadening (denoted ΔE^*) based on an integral rather than a local property of the quasi-elastic line and therefore more sensitive to non-lorentzian line-shape changes in the wings (for details, see Figs 2 and 3).

The variation of ΔE with momentum transfer k is of principal interest here. The significance of k is that the motional properties of the sample (that is, in the present case, the proton self-correlation function) are probed over a scale length of about $2\pi/k$ (Å). Thus, the macroscopic limit of classical diffusion is observed as $k \to 0$, and the behaviour of $\Delta E(k)$ for $k > 1$ to 2 Å^{-1} is dominated by the short-range translational and rotational motions. The detailed form of $\Delta E(k)$ has been investigated both theoretically and experimentally for a variety of dynamic processes[4-6,23]. The simplest case is that of continuous Fick's law diffusion for which $\Delta E = hDk^2/\pi$ where D is the self-diffusion coefficient and h is Planck's constant. The dependence of ΔE on k measured in our experiment is shown in Fig. 3. It is seen, first of all, that $\Delta E(k)$ exhibits an oscillatory structure for both the film stack and the 'fluffy' sample, with a first maximum in the broadening between $k \approx 0.3$ and 1 Å^{-1} at all H_2O hydration levels. This result clearly rules out proton diffusional motions according to Fick's law or any other bulk diffusion process for which the linewidth would increase monotonically with k and then level out at the highest k observed. Also, the effective diffusion coefficients obtained from Fig. 3 as $k^2 \to 0$ fall in the range of 10^{-7}–10^{-8} cm^2 s^{-1}, and this is one or two orders of magnitude smaller than the coefficients of diffusion observed for water in paucimolecular layers or in bulk.

For an ensemble of incoherently scattering particles migrating on a lattice of sites, model calculations[23,24] indicate that linewidth changes similar to those seen in this experiment occur whenever the residence time τ_0 of a particle at a site is large compared with the time during which it moves to a neighbouring site. The qualitative behaviour of the line-broadening results summarised in Fig. 3, if due primarily to incoherent scattering from sorbed water molecules at the protein surface, would therefore suggest water proton motions between discrete hydration sites separated by distances of the order of $1/k_{max}$ where k_{max} is the position of the first maximum in $\Delta E(k)$. The observation of lower k_{max} values for the fluffy sample seems to be consistent with this interpretation. Oscillatory structure of the kind observed here, but with higher k_{max} values, has been widely discussed in the context of neutron scattering from protons diffusing on interstitial lattice sites in metal hydrides[6,23]. Although the migration of water molecules on or near the surface of a protein cannot be expected to conform to any kind of regular lattice of hydration sites, we have nevertheless attempted to fit Chudley–Elliott (CE) jump-diffusion models[24] to the data. Because of the very limited number of data points for $\Delta E(k)$ obtained so far, the curves shown in Fig. 3 and the numbers derived from them must be regarded as illustrative of the technique rather than definitive.

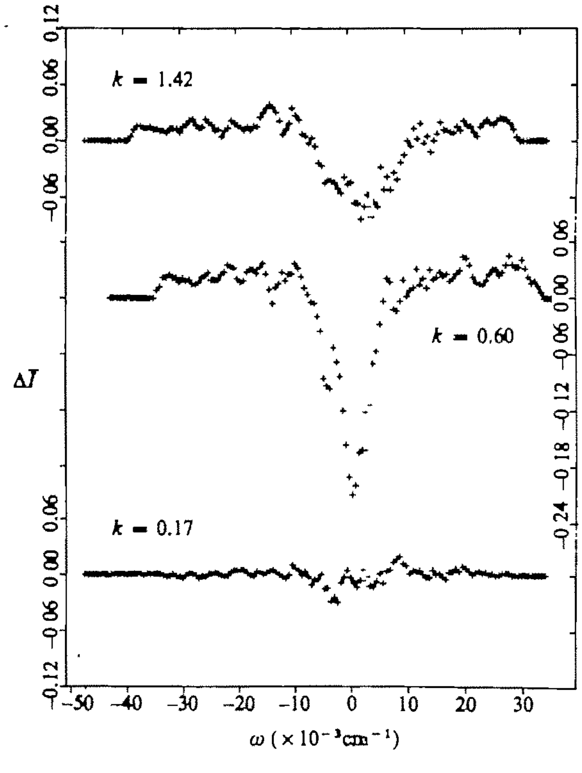

Fig. 2 Wet-minus-dry difference spectra (sample and hydration conditions as in Fig. 1) for momentum transfers k(Å^{-1}) corresponding to $2\theta = 9.8°, 35°, 90°$. For each scattering angle 2θ, the PP-fit to the resolution line $I_r(\omega)$ (dry sample) was peak-normalised such that $I_r(0) = I_{r0} = 1$, then integrated within $\omega = \pm\infty$ to give a reference intensity A_r. The PP-fit to the broadened line $I_s(\omega)$ (wet sample) was integrated and intensity-normalised according to $A_s = A_r$, to give a peak intensity $I_{s0} < 1$. The normalisation factors thus determined were applied to the measured spectra before subtraction. The bottom trace, representing the difference of the essentially unbroadened lines at the lowest scattering angle analysed, demonstrates that overall line shape changes with r.m.s. deviations of less than 2% are observable.

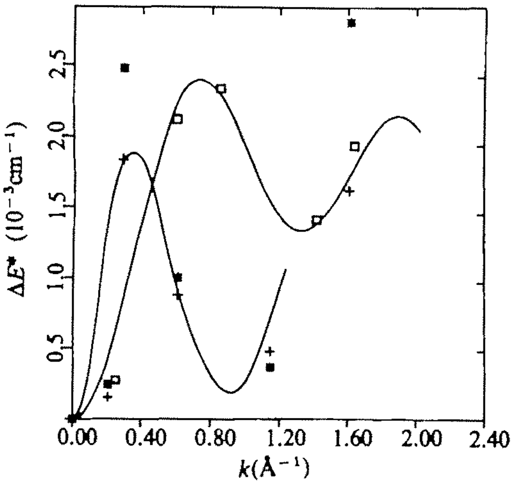

Fig. 3 Quasi-elastic broadenings ΔE^* as a function of momentum transfer k for film stack sample (☐) (hydration conditions as in Fig. 1), 'fluffy' sample at low (+) (RH = 10–30%) and intermediate (∗) (RH = 30–50%) H_2O hydration levels. Resolution lines $I_r(\omega)$ (dry sample) were peak-normalised as in Fig. 2; broadened lines $I_s(\omega)$ (wet sample) were peak-normalised in the same way so that now also $I_s(O) = I_{ro} = 1$ but $A_s > A_r$. Then $\Delta E^* = A_s - A_r$, and for purely Lorentzian profiles, $\Delta E^* = \pi/2(\Delta E_{\text{Lorentz}})$. Comparison with Lorentzian-fitted profiles gave values of 1.8–1.9 for the proportionality factor instead of $\pi/2$, demonstrating the presence of non-lorentzian line shape components.

Except at very low k, the film-stack broadenings agree reasonably well with an isotropic jump model for which $\Delta E \sim 1-(\sin ka/ka)$, the jump distance $a = 5.5$–6 Å and the residence time $\tau_0 = 5$–10 ns. The broadenings measured for the fluffy sample, on the other hand, exhibit a pronounced first minimum at $k = 0.8$–0.9 Å^{-1} that cannot be modelled in any simple way. The lower curve in Fig. 3 represents a weighted superposition of $\Delta E(k)$ resulting from one- and two-dimensional CE models, the reason for this being that at lower hydration levels one might expect the migration of water molecules to proceed preferentially along polypeptide chains at the surface. Here, $a = 7$–9 Å and $\tau_0 = 15$–30 ns. This sample was hydrated continuously from the dry state at a very low rate; the hydration number at the end of the experiment, estimated from the increase in total neutron counts, was 300±50 H_2O per subunit. The number N_r of amino acid residues offering primary hydration sites is difficult to assess because the surface structure and the degree of aggregation of subunits are not known in sufficient detail. Rough estimates would place this number in the range $100 < N_r < 200$ per subunit. The distances d_r corresponding to this range may be evaluated by covering the surface with a hexagonal grid and regarding each vertex point as a potential hydration site. This gives $d_r = 80 \times N_r^{-1/2}$ Å or $7.9 > d_r > 5.6$ Å, that is, a range of values that agrees well with the jump distances deduced from the broadening data by means of simple CE models.

In conclusion, we have presented the first high-resolution neutron spectra showing the interaction of water molecules with a fully covalently deuterated protein. We have observed a hydration-dependent maximum in the quasi-elastic broadening which, at sub-monolayer coverages, seems to be due to the jump-like migration of water molecules closely associated with or bound to the surface of protein molecules. Although more data need to be collected to substantiate this interpretation, as well as theoretical efforts to develop the analysis of quasi-elastic neutron scattering from biopolymers, the results given here demonstrate clearly that details of protein hydration processes down to energy changes of the order of $10^{-5}\,k_BT$ or 10^{-3} cm^{-1} are now within reach of neutron spectroscopic techniques. A very exciting prospect of the contrast variation method is the possibility of experiments aimed at distinguishing dynamically between the water of hydration and intramolecular fluctuations.

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Erratum

In the article 'Model of the fine-grain component of martian soil based on Viking lander data' by Nussinov *et al. Nature* **274,** 859, lines 1 and 2 on page 861 should read: 'can be suspended in the atmosphere, the fact that the mean diameter which can be obtained on this basis is 1–2 μm'. In line 11 on page 861, for 10^4 mol g^{-1} read 10^4 nmol g^{-1}.

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CHRISTMAS BOOKS

Aesthetics and scientific progress

Martin Pollock

Two THOUSAND years ago the distinction we now make between 'Art' and 'Science' would have been meaningless. The so-called 'Two Cultures' would both have been incorporated into 'Ars' and 'τεχνη'. For the past 350 years or so Art and Science have been more sharply differentiated than ever, the latter having developed at an ever-increasing speed to alter our whole interpretation of the Universe and provide technological advances and practical solutions to a large number of our problems (if not to the really vital ones such as how to get on with each other). The edifice of Science is magnificent; yet, somewhat ironically, it has been built up at the expense of increasing restriction in the philosophical concepts on which it rests. "We forget", wrote Whitehead in 1926, "how strained and paradoxical is the view which modern Science imposes on our thoughts". Most of us do not just "forget"; the possibility never even occurs to us. We are blinded by the brilliance of our understanding and the triumphs of our technological achievements.

Nevertheless, within the past few decades, there have been increasing efforts by philosophers, artists and scientists themselves to challenge the assumptions on which Science is traditionally thought to be based and to explore the extent to which the principles and practices of Art and Science respectively may contribute to each other and be seen to share common factors in the forces which drive human beings to express themselves in these two rather different ways. After all, most people are now prepared to agree that there can be beauty, understanding and creative elements in both. In particular, scientists have to extricate themselves from the vicious circle which they themselves create when "what we notice is selected by our concerns and our concerns are excited by what we notice", as Geoffrey Vickers points out in Judith Wechsler's book, *On Aesthetics in Science* (MIT Press: Cambridge, Massachusetts, and London, $12.50; £8.75). The same point, incidentally, was made by Blake 200 years ago when he remarked that "if it were not for the poetic or prophetic character, the philosophic and the experimental would be at the ratio of all things and stand still, unable to do other than repeat the same dull round over again".

To attempt a re-analysis (albeit limited) of Science at a time when its functions in human society and the implications of its achievements are being questioned, is all to the good. Judith Wechsler's contribution has been to edit a collection of six essays by scientists all of whom "maintain that Aesthetics is a crucial factor in the scientific process". She quotes Poincaré in her introduction as saying that "in mathematics, the useful combinations are the most beautiful" and that "it is more important to have beauty in one's equations than to have them fit experiment . . ." (!) (which incidentally calls to mind Watson's and Crick's comment on their structure for DNA being "so pretty that it must be true").

The first two articles in *On Aesthetics in Science* concern the parallels between structural patterns in Art and in Nature. Cyril Smith emphasises the repetitive build-up of modular units to form a spatial hierarchy of recognisable forms in pictorial art and natural systems in physics, chemistry and biology. His description of aesthetic pleasure is "what is left over when what is expected has been cancelled out", which is charming if rather personal. It is, however, surprisingly similar to what Philip Morrison says, in the second article (on "Broken Symmetries") when he maintains that highly satisfying works of art are characterised by "faults in an otherwise regular pattern". Both these essays, stressing the analogy in the significance of similar spatial patterns in Art and Science, are open to the objection that the mind is a unity; it is not artificially divided into an Arts section and a Science section. Some congruency in pattern interpretation is to be expected. The main question is to what extent do these parallels penetrate into the deep structures of the two 'disciplines' so as to have some real significance. This needs much further research before it can begin to be properly tackled.

Arthur Miller gives an interesting (though rather difficult) analysis of the controversies in modern sub-atomic physics in terms of the distress engendered in some physicists by having to face pressures to abandon visualisation in their theorising. It is argued that the wave theory of radiation developed by Schrödinger (and supported eventually, with relief, by Bohr) was inspired by his need to visualise the elements involved; it was opposed by Heisenberg, who preferred non-visual thinking and who thought that Schrödinger's approach was "disgusting". Analogous aesthetically orientated influences operated, so Miller maintains, in the quantum/continuum "antithesis".

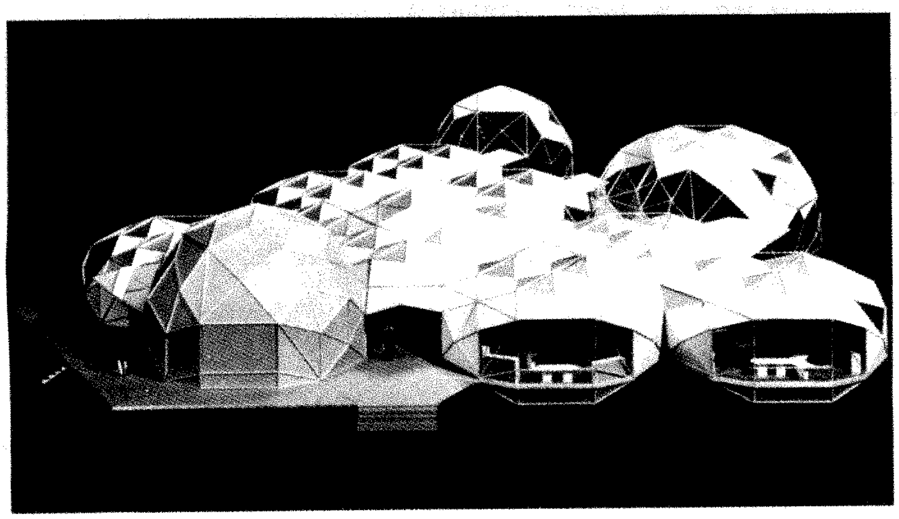
Seymour Papert extends Poincaré's theme that "Aesthetics" (working unconsciously) rather than logic "is the distinguishing feature of the mathematical mind", by analysing sequences in mathematical proofs on the basis of the aesthetic pleasure they can provide ("aesthetic guidance"). It is unfortunate, however, that the principle theorem so presented, being an entrancingly beautiful proof that $\sqrt{2}$ is an irrational number, is desperately confused (at least for the general reader) by a series of shattering misprints on pp112 and 114.

Howard Gruber illustrates what he calls "aesthetic moods" by concentrating on Darwin's evolutionary "trees" and other images used for developing scientific theories. It is the image of a concept that we love, he stresses, not the concept itself. And he rightly points out that there is a beauty in certain types of complexity as well as in simplicity. Indeed, theories do not have to be simple, as some think, in order to be "true" (that is, acceptable); they become simple with familiarity and by the need for simple presentation if they are believed to be true and have to be expounded.

Finally, Geoffrey Vickers develops forcefully and convincingly what is surely one of the most important points of all: namely, that there are indeed two different modes of "knowing" available to the human mind (two "cultures" one might say) based respectively on rationality and intuition, but these do not correspond rigidly, or even approximately, to what we call Science and Art.

The conclusion from this collection of essays is that some fundamental redefinitions and re-thinking are necessary in our approach to Art and Science. Although the pictures presented of the relationship between Aesthetics and Science are inevitably tentative and blurred, the objectives behind the book are excellent. There is a well compiled general bibliography. The articles themselves are provocatively stimulating and, although sometimes inclined to be rather turgid and verbose, are well worth studying by all those not already hopelessly rigidified in their beliefs of what Science is about. We should be grateful to Judith Wechsler for a courageous attempt to freshen and broaden our attitude.

Peter Pearce's well produced and well illustrated book on structural design, *Structure in Nature Is a Strategy for*



Model of school building designed on min-a-max system

Taken from *Structure in Nature is a Strategy for Design*

Design (MIT Press: Cambridge, Massachusetts, and London, $45; £31.50) with its curious and rather misleading title, is in one sense a formal geometrical analysis of the hierarchy of natural and man-made structures considered by Smith and Morrison in Wechsler's book. But it is really only tangentially relevant to the principles of Aesthetics as applied to Science and technology. For the general reader it is often rather heavy going. It is an exhaustive (and at times somewhat exhausting) compendium of different types of space-filling polyhedra based on a limited number of simple different polygonal surface structures, pieced together in a variety of ways. The objectives in design, both in natural and man-made structures, are—and must be—economy of energy, effort and cost (closest packing of a minimal number of different types of constructional 'unit') combined with optimal efficiency (strength for resisting internal and external strains) and maximal diversity (to meet a wide range of different environmental requirements). This leads to numerous structural similarities between natural patterns (snow-flakes, soap bubbles, honeycombs, pineapple skin, cracked mud, giraffe skin markings) and man-made structures (for example, the famous Buckmaster Fuller 'geodesic dome') according to the exigencies of different situations.

One of the more significant points emphasised by Pearce is the superiority in strength and stability of the triangle over the square as a unit side for complex polyhedra subject to various strains and stresses. This results in a series of three-dimensional structures in which the right-angle occurs relatively rarely and walls and ceilings, as we know them, have almost disappeared. The box-like construction of most contemporary architecture is thus largely abandoned. Nor are there any 'real' curves—except in so far as the so-called 'saddle polyhedra' are involved, these having sides of minimal surface area formed when the unit polygon is skew (that is, non-planar). With special properties of strength and stability, they are involved in the construction of tunnels, continuous surfaces and solidly enclosed labyrinths.

A huge range of different polyhedra is described systematically in terms of their constituent elements, illustrated by a vast number of diagrams and photographs, which will make most ordinary readers dizzy trying to work out what a monster composed, for instance, of hemi-saddle digonal disphenoids interdigitating with octahedrally truncated rhombic dodecahedra would actually be like. Yet this could, no doubt, be of great value to the experimentally minded architect really trying to break new ground in functionally improved design and cheaper construction costs.

Pearce is very production-conscious and has his own company, *Synestructics*, for developing his ideas which range from children's playground toys to homes, schools and factories. He has built many models, though, so far, no full-scale constructions. His buildings bristle with angles and slopes in all directions. They look fascinating and some (though certainly not all) are rather beautiful. Most appear bizarre to the conventional eye. In spite of the theoretical diversity available, one might suppose, from all the permutations and combinations possible of standard unit building elements provided (nodes for a variety of fixed angles, triangular plates of different sizes and shapes with opaque or transparent panels, and so on), the completed structures do not appear as different from each other as one might expect—probably because our orthogonally prejudiced eyes are blinded by all those angles. But they may indeed represent at least one important trend in the architecture of the future. Some of the constructional principles are surely excellent; so indeed is the attitude towards economy of cost and effort. But the basic Aesthetics behind these designs is elusive probably I think because no real attempt has been made to develop or expound it—if indeed it really exists. The practical elimination of the monumental curve seems to impoverish many of the designs and the suppression of the right-angle introduces a sensation of insecurity. But such reactions are so often based on personal and fashionable prejudice. To many of us, Pearce's ideas are unfamiliar, exciting and original. They surely deserve further exploration at a practical level. ☐

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Metaphor of the mechanical body

The Body in Question. By Jonathan Miller. Pp. 352. (Jonathan Cape: London, 1978.) £7.95.

THE medieval commentator and teacher, faced with a previously unknown book and attempting to understand it himself and explain it to others, asked of it a formal rote of questions designed to relate it to the known intellectual world. Sometimes the modern reviewer is in the same position when attempting to relate the book in question to the particular interests and expectations of the readers of the journal for which he is writing.

To explain the *Body in Question* our scholastic predecessors would undoubtedly have thought it necessary to bring into play the whole apparatus of Aristotelian causality which, given ingenuity, could explain anything: the efficient cause of the book is Jonathan Miller, intelligent and charming physician, erstwhile historian of medicine, and arch-communicator; the final cause of the book is the opportunity to take advantage of the advertising furnished by a related 13-part television series; and the material cause is some two and a half pounds of glossy paper. The remaining cause is the formal: the blueprint in the author's mind, translated into the format and content of the book.

The author at once tells us what the book is not: neither a history of medicine nor a complete survey of physiology, and it remains the reviewer's job to tell the reader what the book is. It is an attempt to use historical and modern evidence to present physiology in terms of physical science. To put it another way, it is pop physiology with history to tell us how the metaphor of the body as a machine came true—"the story of the identification of the machine within the ghost". This is a serious argument and it deserves serious review. Let us look at the history.

Perhaps the best feature of the book is the discussion of the limits of perception: how the brain's past experience leads it to impose a pattern on the data of perception, enables it to choose between alternative perceptual interpretations, and how it distorts perception by being "theory laden". The author shows clearly that this process has in the past led to "false" scientific ideas, and this is a hopeful start to the historical problem of reductionism or mechanism. But the brain distorts historical as well as scientific knowledge, and although it may be tempting for the physician to argue that "[Modern] Natural science is the native idiom of the brain made explicit", such a statement leads to a historically suspect endorsement of Miller's whiggish main thesis, that the true, mechanical, account of the body emerged only in the last century. It is not true that the medicine of the Middle Ages was characterised by "monkish superstitions", and it is not true that eighteenth century medicine was a "tissue of contradictions" with "no consistent intellectual standards and no organised body of scientific principles", or that its practitioners were "unable to make sense" of the doctrine of irritability. Miller holds that at last by the 1830s "the medical profession was almost within reach of coherent scientific credibility", but in fact it was precisely credibility of this sort that made Riolan oppose Harvey, and the College of Physicians of London oppose reform, two centuries before.

To support his argument about the development of the idea of the mechanical body, Miller naturally looks at the historical use of mechanical analogy (not metaphor) by medical writers, particularly in relation to involuntary motion and the beat of the heart. This again is a serious historical argument and the influence of the mechanical pump on Harvey's ideas has been seriously discussed by historians: it was probably negligible in *De Motu Cordis*, but it is much more likely that Harvey was influenced by the notion of a valve as illustrated by the analogy of sluice gates. This kind of argument needs very close examination of the sources, and Miller's examination of Harvey and the circulation is unsatisfactory.

Fifteen hundred years before, Galen had used the analogy of a suction-pump, and Erasistratus, an ancient even to Galen, thought the heart was a force pump, as Harvey well knew. The descriptions and relationships of Galen's physiological system, Harvey's, and mechanical analogy, are full of errors. Apart from technical mistakes, to call Galen's system "an industrial plant halfway between a brewery and a blast furnace" is to grossly exaggerate Galen's use of mechanical analogy, and to say that Harvey's quantitative arguments mark him as the "first modern biologist", who took "an altogether mechanical view of the function of the heart" and who wrote a book "with the force of great literature" is to be blinkered by twentieth-century hindsight to Harvey's great and constructive conservatism.

The author's argument is further supported by a great number of illustrations often so striking as to rival the text for the reader's attention. A dramatic, if hardly beautiful, double spread of a new born infant illustrates only that "The possession of body may be a necessary condition of being a person but it is not a sufficient one"; an illustration of a pot being turned has the solemn caption "Pottery is a typical way of making things happen . . ." and supports only a description of the role, or lack of a role of the hands in faith-healing. Is not a photograph of a large man chasing a naked boy down a dark corridor a gratuitous illustration of "A nervous system which can recognise and react to threats as and when they occur"? The technique is pure television, frozen on to the glossy page, and the reader emerges from the book like an after-dinner viewer switching off a cultural documentary with a faint feeling that his mind has been improved, but unable to quite remember the intellectual meat in the very professional presentation. No biologist will be surprised by the ideas in the book, but the layman will find that it does tell him a large number of interesting things about his own body.

R. K. French

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Tree appreciation

It is not surprising that the ravages of Dutch elm disease should have stimulated an attempt to produce a compilation properly celebrating the role of elms in our landscape and culture. The result of such an attempt is Gerald Wilkinson's much promoted *Epitaph for the Elm* (Hutchinson: London, £6.95). It begins with chapters on the elm in poetry and painting; but whereas the poets—particularly Clare, Edward Thomas and Betjeman—are well represented, the painters have not fared so well. Constable is featured prominently with some fine illustrations—but also some poor ones. One whole page is devoted to a postcard size view of Dedham Vale in which hardly any trees can be seen. More recent landscape painters such as John and Paul Nash have been undeservedly ignored.

The next section deals with the botany of the elm, and forty pages (a quarter of the book) are devoted to the genus *Ulmus*. Unfortunately Mr Wilkinson proves to be an inadequate guide through the labyrinth of elm taxonomy. The most widely held view today is that the Wych elm (*U. glabra*) is our only native elm, and that in the pre-Roman period there was a series of introductions to Britain of various clones of the European field elm (*U. carpinifolia*) and of *U. carpinifolia* × *U. glabra* hybrids. Other hybrids are thought to have subsequently been formed in this country. This view, based on the meticulous and scholarly research of R. H. Richens, receives no attention from Mr Wilkinson. Rather, he prefers to describe in great detail the older work of R. Melville in which a number of elm types were described as distinct native species, and he waxes very indignant that the recent editions of Clapham, Tutin and Warburg do not accept them. One's doubts as to whether Mr Wilkinson could recognise some of these elm 'species' himself are reinforced when, in dealing with the Dutch elm, that distinctive clone of southern England, he states "I have not been able to satisfy myself that [this elm] exists as a recognisable type in the English countryside".

English elm

Photo: Forestry Commission

His confusing treatment of our elm population leads him to make some very questionable statements in the next chapter, that on the elm in prehistoric Britain. It is followed however by a much better chapter on the historical period, with quotations from John Aubrey, John Evelyn and others, and by nicely illustrated sections on the past and present uses of elm wood.

Sadly, however, the book ends with a very weak section on Dutch elm disease. It is clear the Mr Wilkinson has a very imperfect understanding of the biology of the disease, and this leads him to make wild remarks such as, "even though elm bark beetles are not supposed to lay their eggs in shoots of less than ten years of age some of the pollarded elms in Suffolk are dead".

In summary, despite the fact that it is attractively presented and contains some good photographs, *Epitaph for the Elm* is too flawed to be an adequate tribute.

The Dutch elm disease epidemic is undoubtedly at least partly responsible for the current interest in trees of all kinds, and this year two new guide books have appeared. Roger Phillips' *Trees in Britain, Europe and North America* (Pan: London, paperback £3.95) is a photographic guide to "500 of the most common and important trees of the temperate climatic region". It is aimed at people with no botanical training and consists of two parts: the first comprising fifty pages of leaf photographs from which the initial identification is made; the second consisting of brief descriptions of the trees in alphabetical order, illustrated with photographs of the flowers and fruit. The "leaf index" should prove quite useful, although some of the photographs are blurred and most of the names on the first page have been transposed. The photographs of the flowers and fruit are very attractive, although here again there are mistakes which indicate that the final stages of publication were over-hurried. Nevertheless this is a book which many will find helpful and enjoyable.

The late Herbert Edlin's *The Tree Key* (Warne: London, hardback £5.95; paperback £3.95) relies on paintings rather than photographs, and there are pleasing pictures showing tree shape, summer and autumn foliage, leaves, flowers, fruit and seedlings. The main problem is that the book has the formidable ambition of being "a really practical guide for those seeking trees on either side of the Atlantic". As it is pocket-size this means that, for most trees, identification can only be to the generic level. Oddly, in view of the name, there is no key in the sense that one can systematically track down any particular tree. There is a series of classifications (leaves on the basis of shape, soft fruit on the basis of colour) but they are incomplete. Thus one cannot find *Catalpa* listed among the genera possessing "fruits with many seeds".

No doubt there are financial attractions in producing a book that can be sold on both sides of the Atlantic, but I wonder if this is really a sound policy, particularly with such a small book as *The Tree Key*. It means that some of the very limited space is devoted to species such as the Rock elm (*U. thomasii*), that are almost non-existent in Europe.

Trees by Andreas Feininger (Penguin: Harmondsworth, UK, paperback £3.95) is described by its author as "a tree appreciation book, rather than a guide or picture book". Sample chapter headings indicate its approach: "The oldest living things", "Leaves", and "Fall colours". It is illustrated by 160 photographs, mostly taken in North America but also some in Europe. Many of these are beautiful and evocative, in particular those of Californian trees such as the Redwoods and the Bristle-cone pine. There are also some pleasing black-and-white pictures of bark and weathered wood. The accompanying text of some 100 pages is lucid if slightly repetitive.

Mr Feininger's great interest in the conservation of the North American forests comes through strongly, and the book ends with a section—not very appropriate for British readers—on conservation societies in the USA. The text seems to be unchanged from the original American edition of 1968 so that some of the facts, figures and attitudes are a little dated. For example, he states that in the USA consumption of wood for fuel has declined steadily through the past century, whereas the past-five years must have seen a marked increase in its use for this purpose. **John N. Gibbs**

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Nature's traces

PICTURES add a vital dimension to books on natural history and, with the improvement in techniques for printing illustrative material, more and more reasonably-priced illustrated biology books are coming on to the market.

Nature Detective by Hugh Falkus (Gollancz: London, £6.95) is a most handsomely produced book with many excellent black-and-white photographs. Many of these are by Niko Tinbergen, whose knowledge and enthusiasm have obviously been a major source of inspiration to the author. The pictures are the most important feature of the book and illustrate very clearly the insights that can be gained into the behaviour and ecology of animals by careful examination of the signs, tracks and droppings that they leave behind. The text provides some background information about the animals and localities illustrated.

David Stephen's *Guide to Watching Wildlife* (Collins: London, £1.75) is a paperback which gives advice on where, when and how to watch birds and mammals by day and by night. It is richly illustrated with the author's own drawings and photographs, the latter testifying to Mr Stephen's skill in practising what he preaches. The emphasis of the book is on practical skills, such as how to watch badgers, and it provides a very useful guide for those who wish to explore nature more actively.

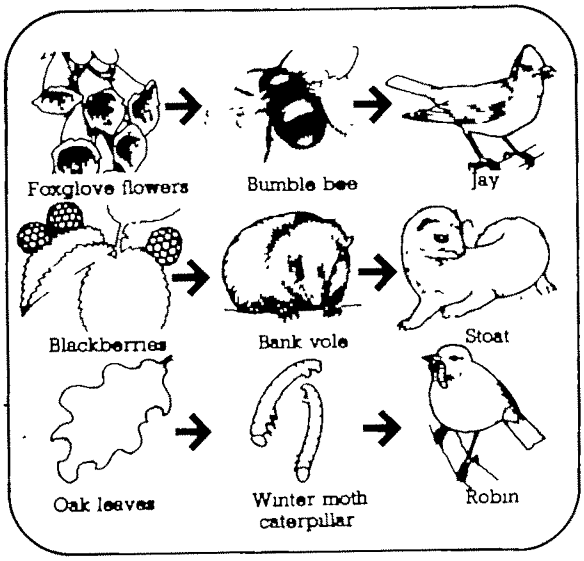
Recording Natural History Sounds by Richard Margoschis (Print and Press Services: Barnet, London, UK) is a very specialised paperback book suitable for a handful of enthusiasts and for researchers working on animal sounds. It contains much detail on principles and techniques and a number of photographs which are not generally very informative, many of them simply showing various types of tape recorder.

Nature Day and Night by Richard Adams (Kestrel. London, £3.95) is an attractively produced book, but one whose purpose is not very clear. Its theme is the way in which animals and plants are adapted to the alternation of day and night. The book has two rather different components. Richard Adams contributes a series of essays on, for example, "the meadow by day"; these tend to be rather whimsical in style. Max Hooper has written a series of scientific essays on specific subjects such as biological clocks, and David Goddard provides attractive illustrations, those for the general essays being double-page spreads crammed with animals and plants, sadly many of them too small for accurate identification. The scientific sections are excellently illustrated with drawings and diagrams and are the more successful aspect of this book.

Exploring Nature by David Hall (Hamlyn: Feltham, UK, 50p) is a practical guide for children and seeks to encourage active investigation and experimentation. Projects discussed include setting up aquaria, trapping soil animals, making spore prints from fungi and investigating photosynthesis. There is a wealth of ideas which should not only stimulate enquiring youngsters but will also be a useful stimulus to their parents and teachers.

Another children's book, *The Young Naturalist's Guide to Conservation* by Neil Arnold (Ward Lock: London, £2.95), has the admirable aim of providing practical suggestions for young people wishing to protect and encourage wildlife in their immediate surroundings. It describes how to maintain ponds, plant trees and observe birds and other wildlife. The text is concise and clear but the illustrations are somewhat variable in quality.

The *Chatto Nature Guides* (Chatto and Windus: London, hardback £4.50; paperback £1.95) are translated from German books and are illustrated by colour photographs. Each species described is covered by a photograph and a brief text giving details of characteristics, habitat and distribution. Individual titles are ***Birds*, *Wild Flowers*, *Mushrooms and Fungi*, *Insects*, *Aquarium Fishes*** and ***Minerals and Rocks***. The photographs are generally excellent, though in some instances are not suitable for the purposes of identification in the field. For example, the Cuckoo is illustrated by a photograph of a fledgling which lacks the characteristic long tail of the adult At such a low price one could not expect these little books to be comprehensive in their coverage, but there are some very serious omissions. For example, in *Birds* there is no Mistle Thrush, Lesser Black-backed Gull, Kittiwake, Dunlin or Shelduck. Perhaps these deficiencies reflect the German origin of these books; the volume on birds is notably short on coastal species. A good feature of a number of these guides is that they draw attention to instances where species may be confused with one another and emphasise the points of difference. *Mushrooms and Fungi* includes a clear code to indicate which species are palatable and which are poisonous. *Aquarium Fishes* includes hints on how each species



Woodland Food Chain—Nature at Work

Nature at Work

Introducing Ecology

This beautifully illustrated book introduces some basic concepts of ecology and interprets the relationships between plants and animals in terms of their roles as producers and consumers of energy. The plants and animals around us are constantly interacting with each other and with their non-living surroundings to form an intricate network of activity which is illustrated throughout this book with the aid of examples from two familiar ecosystems, a woodland and a rocky seashore. The book demonstrates how these two ecosystems work by following the energy flow as it is captured by plants and transferred to other living things as they feed. This account is written for the general reader who wishes to understand more about the living world. The lively and stimulating text is accompanied by many vivid and colourful illustrations specially commissioned for the book.

The book has been prepared by the staff of the British Museum (Natural History) and coincides with a major new exhibition at the Museum.

A joint publication between Cambridge University Press and the British Museum (Natural History).

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Endurance of Life

The Implications of Genetics for Human Life

MACFARLANE BURNET

This is an important book in which the author, one of the most distinguished biomedical scientists of the twentieth century, examines the phenomena of old age and presents an original theory of human ageing in terms of genetics. The central hypothesis is that the changes associated with age arise, for the most part, from the accumulation of genetic errors in almost all cells of the body. The author goes on to discuss the genetic factors which, moderated by the environment, produce intelligence, temperament and behaviour. In the final chapters he discusses the genetic inheritance of modern man and the difficulties it presents in today's complex civilisation. The author suggests that antisocial behaviour is caused not simply by environment, but largely by the genes. The author has founded his argument in scientific fact, but as a pioneer in an uncharted area, he relies on analogy and speculation. The result is a stimulating and controversial work on issues which concern and touch on the lives of all human beings.

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William Henry Bragg

1862-1942 Man and Scientist

GWENDOLEN M. CAROE

This biography of Sir William (Henry) Bragg is written by his daughter for the general reader and tells the life story of an eminent scientist who became a spokesman for science during the first half of the century. Sir William (Henry) Bragg and his son Sir (William) Lawrence Bragg were the first to explore the structure of crystals, using X-ray diffraction techniques, and their work was recognised by a shared Nobel Prize in 1915. There are extensive quotations from Bragg's own notes about his early life which are fine prose in themselves and have lively relevance to the present day.

'She (the author) has brought to life a big slice of those heady days when science—and physics in particular—leapt forward into atomic structures, with X-rays and radioactivity, crystallography, J. J. Thomson, Einstein and Planck.'

The Economist

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INTERNATIONAL JOURNAL OF INVERTEBRATE REPRODUCTION

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Aims and Scope

The International Journal of Invertebrate Reproduction will be devoted to the rapid publication of complete papers and short communications in the following fields of sexual, reproductive and developmental (embryonic and postembryonic) biology of the Invertebrata (excluding the Protozoa). The journal will publish only papers of high scientific quality and originality, shedding new light on fundamental principles and processes. In addition, it will provide the means for better communication between researchers by the publication of penetrating and provocative minireviews, announcements, reports of meetings, news and views, notices, and book reviews.

The quality of the journal is reflected in the following list of papers included in the first issue:

R.P. Ruegg and K.G. Davey *(Canada)* The effect of C18 juvenile hormone and Altosid on the efficiency of egg production in *Rhodnius prolixus.*

D.J. Wildish *(U.K.)* Reproductive consequences of the terrestrial habit in *Orchestria* (Crustacea: Amphipoda).

D.F. Went *(Switzerland)* Pathogenesis in the dipteran insect *Heteropeza pygmaea):* an interpretation.

Uma and T. Subramoniam *(India)* Histochemical characteristics of spermatophore layers of *Scylla serrata* (Forskal) (Decapoda: Portunidae).

M. de Jong-Brink, J.P. ter Borg, M.J.M. Bergamin-Sassen and H.H. Boer *(The Netherlands)* Histology and histochemistry of the reproductive tracts of the pulmonate snail, *Bulinus truncatus,* with observations on the effects of castration on its growth and histology.

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should be kept in the aquarium.

The *Penguin Nature Guides* (Penguin: Harmondsworth, UK, hardback, £1.95) have also been translated into English, from either Danish or Swedish. *Fishes of the British and Northern European Seas* has excellent illustrations by Bente Nyström to aid identification of species. There is also much interesting background information on fish and fisheries illustrated by diagrams and photographs. *Birds of Wood, Park and Garden* and *Birds of Sea and Coast* conform to the now familiar fieldguide format with excellent paintings by Lars Jonsson in which the birds look very much alive, a brief text on their natural history and characteristics, and distribution maps. *Plant Communities* incorporates the interesting idea of arranging plant species in the communities to which they belong. Separate sections deal with meadows, the sea shore, oak forest, and so on. A problem with this book is that (as is made clear in the book) many of the species shown do not occur in Britain. This is a problem inherent in books translated from another language. Also this book shows very few species and is thus of limited use as a field guide. Finally, *Fungi of Northern Europe, 1: Larger Fungi* and *Fungi of Northern Europe, 2: Gill Fungi* have excellent illustrations, among the virtues of which is that they show much of the variety of form which each species can achieve. These attractively produced books can be recommended as a reasonably priced, exceptionally well illustrated and informative series.

T. R. Halliday

T. R. Halliday is Lecturer in the Department of Biology at the Open University, Milton Keynes, UK.

Solving the problem of bird identity

THE world shrinks for the bird-watcher—and others—as 'special interest' package tours provide inexpensive ways to view new continents, where we discover birds of new families and orders of which perhaps we had hardly imagined the existence; and where we can often see familiar species in strange and exotic habitats. The television too takes its viewers to wild lands and curious creatures. Whatever way we travel, even if it is only by reading travel books, we need a source to tell us something of the characteristics and relationships of some beautiful bird seen hazily in the hot desert or all too briefly in the rain forest.

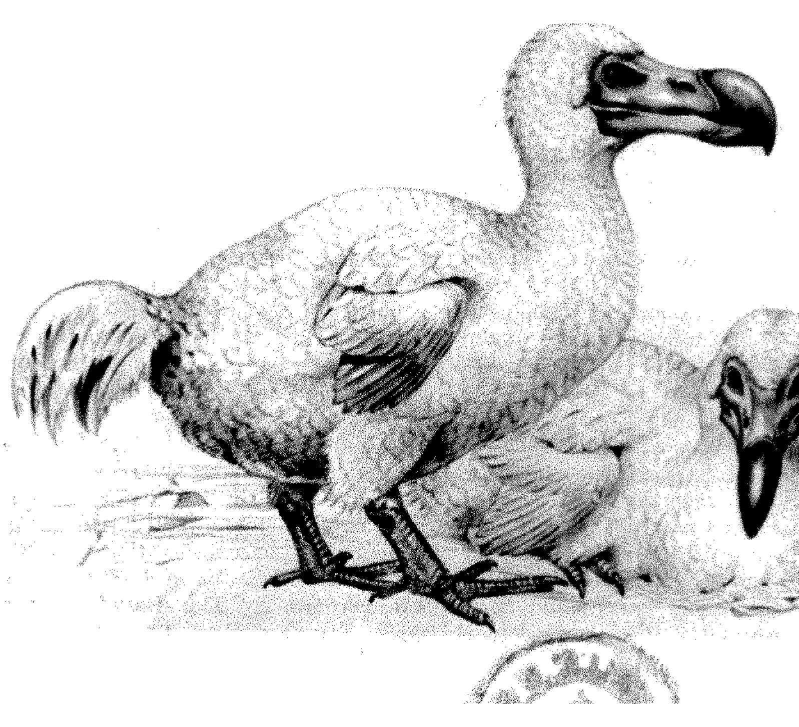
There are no fully comprehensive books on birds of the world other than checklists, and Dr Colin Harrison's *Bird Families of the World* (Phaidon: Oxford, £9.95) is probably the nearest that we can find at this price to such a survey of the families of birds existing in the world today (together with some extinct ones). Each family account is written and signed by one of 43 specialists and describes the numbers of species and genera in each family as well as noting the main features of their distribution, feeding behaviour, and nesting habits. About 900 species are illustrated by useful and accurate coloured drawings by Ad Cameron. Dr Harrison had used J. L. Peters' order for arranging his material but reminds the reader that there is another possibility and points out the alternative names and orders where they could be applied. The book is a 'popular' but serious and well-illustrated attempt to convey a picture of the main features of the birds of the world.

We don't have to travel far from home to have identification problems; moving from a world range to the identification of birds in Britain John Andrews has written *Birds* which is one of the *Nature Guides* published by Hamlyn (Feltham, UK, £1.50) and designed for the many people who wish to name easily and confidently the commoner conspicuous birds they see in their gardens, in the countryside and on their holidays. Just over 200 different species are described but only 168 are illustrated by coloured photographs. Whether colour photographs are adequate for the purpose of identification is always debated; in this pocket-sized volume most photographs do their job. But the photographs of the harriers are not really clear enough and it would have been helpful to have been told the sex of the Serin and the pied wagtail—or is it a white wagtail? Also, is the bird which is labelled great black-backed gull really a great black-backed? The contrast between the grey of the mantle and the black of the primaries as well as the colour of the legs as shown in my copy would lead me to think that it it a lesser-black-backed gull. Which all goes to show that photographs can be difficult.

John Andrews introduces his book with several paragraphs on the points to look out for when identifying birds. He gives a valuable table of habitats in which birds can be found and a short description of each species opposite each photograph. The book would make a useful Christmas present for someone, child or adult, beginning to identify birds but who does not wish to be confused by the large number of choices provided by the more advanced Field Guides.

Turning from problems of identification to other aspects of ornithology Eric Simms has written his second volume in the *New Naturalist* series, *British Thrushes* (Collins: London, £6.50), which describes some of the most familiar birds in the British Isles and in Europe. Within his compass he manages to incorporate not only the common resident thrushes, such as song and mistle thrush, blackbird and ring ouzel and the winter visitors, redwing and field fare—which also nest in very small numbers in Scotland—but also information about the thrush-like chats, stonechat, whinchat, wheatear and robin as well as these exciting vagrants which occasionally visit us from the distant north-east.

The text is illustrated by many excellent black-and-white photographs, by Robert Gillmor's pen-and-ink drawings of the displays of the different species, and by distribution maps, sonagrams, histograms and additional data supplied by experts from the British Trust for Ornithology and other organisations. Indeed the quality of the information makes this a very comprehensive volume and one which all interested in birds should have. The book, which should become a classic, is a pleasure to handle and dip into; compared with many other books produced these days it is very good value for money.



Réunion solitaire (*Raphus solitarius*), a species of dodo

Taken from *Bird Families of the World*

Guides to identify birds or their eggs are common enough and guides to bird-watching places are becoming commoner. The danger of this type of guide is that it will attract far too many people to the more delicate areas, disturb the birds and thus destroy the places they have come to see. Eric Hardy in his *Guide to the Birds of Scotland* (Constable: London, £3.95) has provided a pretty comprehensive list of places and a more comprehensive one of birds that appear in various parts of Scotland, but the bird list is rather mixed-up and makes very difficult reading. He has, however, been forbearing—careful to avoid mentioning some of the places where pressure by bird-watchers could cause big problems—and the regional maps show sufficient areas of high ornithological interest to keep bird-watchers busy for many holidays. But it is a pity that his obsessional hatred of the 'establishment' and particularly the Royal Society for the Protection of Birds, provides such a sadly sour introduction to a book which should be leading us to a greater enjoyment of birds.

While on the problems of identifying birds and their habitats, I must mention briefly another book by Dr Colin Harrison entitled *A Field Guide to the Nests, Eggs and Nestlings of North American Birds* (Collins: London, £6.50), which illustrates 622 eggs and 147 nestlings in colour. Although it is a textbook or field guide, Dr Harrison emphasises that it is for bird-lovers rather than egg collectors. For birds on the North American continent from the Arctic to the southern boundary of the USA, it incorporates basic information on habitat, nest, eggs, breeding season, incubation, nestling period, and so on. It may not appeal to the average British bird-watcher but in the United States it should become a standard work of reference.

Identification is but a first step to a deeper interest in birds and *The World of Birds* (Sampson Low: Maidenhead, UK, £3.95; the Italian author's name, Gianfranco Bologna, is rather hidden away) is the book in this collection which attempts to interest the beginner in what birds actually do. The title is rather a common one and it, or one rather like it, has been used for four or five books in recent years. What Bologna is really writing about is the way in which birds live. Many of the examples he chooses of bird biology and behaviour are common to many other recently published books on this subject and his short list of references are virtually identical to the others. What perhaps distinguishes the book is that he has used the experience of Italian authorities to supplement the standard examples. Quite clearly this book is a translation and occasionally his translator has let him down—once by referring to the "hibernation" area of the ringed plover. It is not a technical book and it could help the bird-watcher on his way to becoming an ornithologist.

The emphasis of the last two books switches from the scientific aspects of birds to the artistic. Robert Dougall in his new book *A Celebration of Birds* (Collins: London, £5.95) has left the television scene to indulge in his retirement hobby of bird-watching. He has collected together some of his own ornithological reminiscences and his favourite ornithological verse. The chapters are centred on 15 species most of which are common enough, like the robin, blue tit and sparrows, but he also goes to the other extreme and writes about the avocet and osprey. This book does not pretend to be an ornithological treatise nor a complete collection of bird verse, but rather a book for Dougall addicts with an ornithological slant. It is illustrated by drawings, some black-and-white, and some in colour.

For a selection of 36 bird portraits painted by C. F. Tunnicliffe accompanied by a practical text written by Linda Bennett, we have the *RSPB Book of Garden Birds* (Hamlyn: Feltham, UK, £2.95). The original paintings were commissioned by the RSPB as front covers for its old magazine *Bird Notes* or as Christmas cards, and in this book one finds a very good portfolio of Tunnicliffe paintings for a very low price. The main purpose of the book, however, is the practical text, describing the factors which make gardens attractive to birds, as well as the history of human interest in those bird gardens where bullfinches might even be encouraged to take fruit. It brings to mind one of the earliest practical books on bird gardening written by the Baron von Berlepsch, which still has a lot to offer. Linda Bennett gives good advice on the art of bird gardening and of course Tunnicliffe's paintings are always a delight. It would make a very attractive Christmas present.

Peter Conder

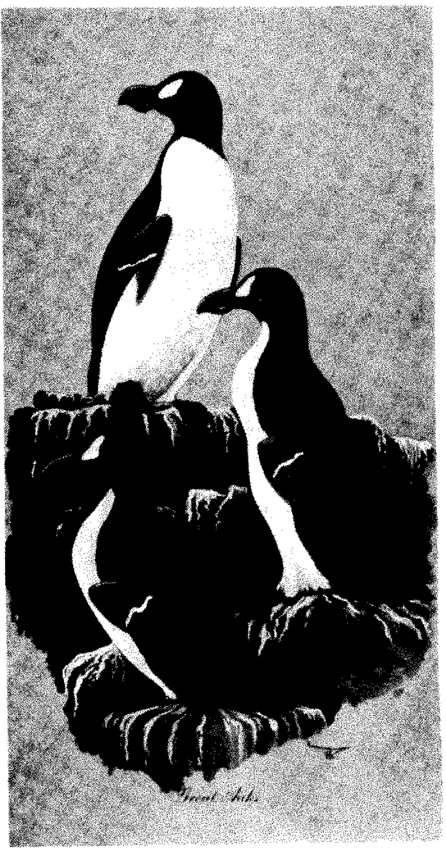
Peter Conder edits the 'News and Comment' section of the journal British Birds, *and acts as a consultant on behalf of the IUCN.*

Contributions to conservation

It would be pleasant to be able to believe that the great number of people who now support nature conservation organisations in Britain and in a few other countries do so out of commitment born of knowledge. In fact, the vast majority have joined for entertainment and, perhaps, out of a vague sense of concern. Not that there is anything wrong with that but it is important that the extent and nature of their involvement is recognised. At present, the statutory and voluntary conservation bodies operate in rather favourable conditions—private affluence and leisure meaning more public interest and support, and the national economic position inhibiting some demands on the natural environment. When times really do grow hard—as it seems they must—will the support melt away just when it is most needed? Logically, the answer must be "Yes" unless in the, perhaps brief, interim every effort is made to develop people's appreciation of nature, their understanding of the complex issues that lie at the roots of conservation and, thus, their commitment.

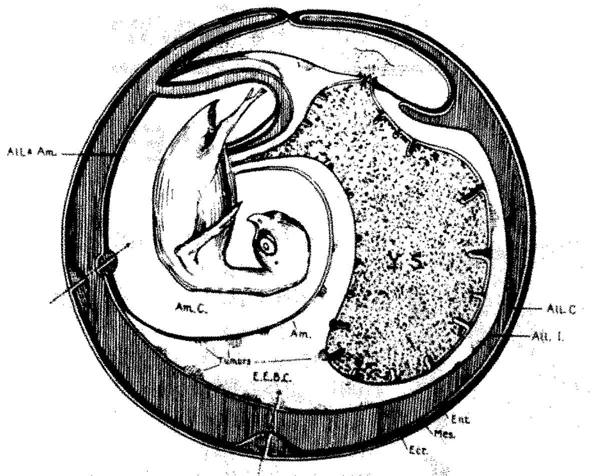
Any book or article, broadcast or film which starts the amateur naturalist along the road of understanding how man's activities affect wildlife is contributing to conservation. *Vanishing Birds: Their Natural History and Conservation* by Tim Halliday (Sidgwick and Jackson: London, £7.50) certainly comes into that category, treating a fairly difficult subject in a straightforward way but without avoiding the key issues.

Commencing with a general summary of avian evolution and strategies for reproduction and survival, the book examines, both in general terms and with case studies, the effect of man's past and present activities on bird populations. It is overwhelmingly clear



Great Auks

Taken from *Vanishing Birds*



The development of the chick—An Introduction to the History of Virology

An Introduction to the History of Virology

A. P. WATERSON and LISE WILKINSON

This book describes the development of knowledge about the study of viruses and the diseases which they cause in plants, animals and man. Towards the end of the nineteenth century it became clear that some diseases were caused by infectious agents which differed from bacteria, but it was not until the middle of the twentieth century that a clear view of the biochemical basis of viruses emerged. The authors have attempted to see viruses through the eyes of those experimenting with them at the time, and in particular to assess their concepts of the material they were working on. It is essentially the story of ideas and of their origins, and of the formulation of the virus concept to its present-day clarity. In addition, it throws interesting light on how a scientific discipline like virology emerges as a subject separate from a larger field. The book contains much about the neighbouring fields of protein chemistry and cell biology.

'The first page catches the reader's eye by a magnificent colour plate of a bunch of variegated tulips, which has distinctive patterns attributable to an infection by a virus, the tobacco mosaic virus. From here onwards the reader will be hooked by the dazzling story of virology (many of the founder fathers are with us still) ably unfolded.'

The Lancet

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A. V. WATSON

This lucid introduction to British mosses and liverworts, first published in 1955 and now in its second edition, has become a standard work. The author gives full descriptions and ecological details of over 200 of the commoner and more notable species, illustrating each to show its major features. The introduction on the general characteristics of bryophytes, and the simple terminology enable a beginner to use the book without difficulty; the key and habitat lists will be invaluable to beginners and specialists alike. In the second edition there is a complete rearrangement of the liverworts to follow the current check list, a revision of the keys to both mosses and liverworts, and the inclusion of a short bibliography. In addition, there are some new illustrated accounts of the main species and species not listed before.

'It is warmly to be recommended.'

Journal of Ecology

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Science

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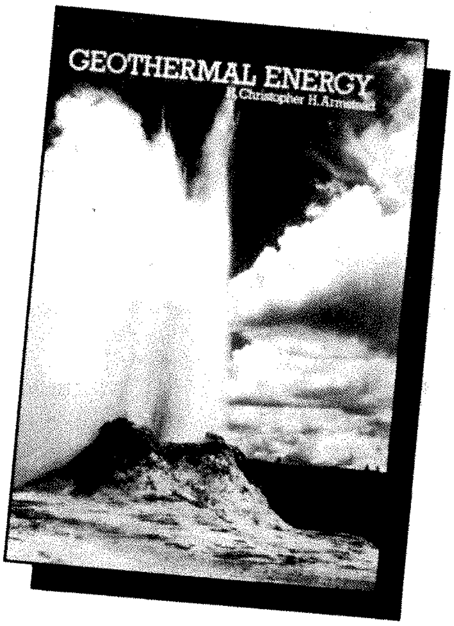

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WITH PARTICULAR REFERENCE TO INSECT POPULATIONS
Second Edition
T. R. E. SOUTHWOOD F.R.S.

The first edition has become a regular and well worn text on ecologists bookshelves throughout the world. This new edition represents a substantial revision – a number of chapters have been completely rewritten, others modified to incorporate advances in practice and theory made in the last twelve years.

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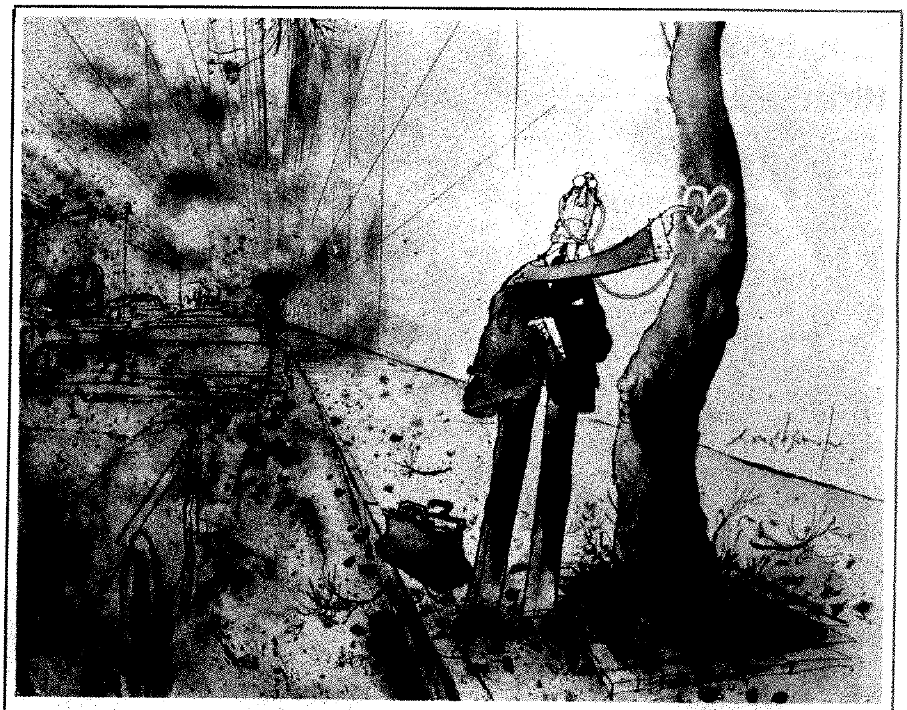
that, despite a few advances, the natural environment is exploited as energetically as ever it was—often without the justification of ignorance or great need. We deplore the feckless extermination of the great auk, not only for food but as a source of feathers: to loosen their plumage the birds were dropped into vats of boiling water, heated by burning the oily corpses of their fellows. Yet we effectively ignore the fact that the only woods in which Abbot's booby breeds are being felled in order to mine phosphates. And who protests at the stupid and criminal destruction of Amazonian forests, with their indigenous wildlife and defenceless human communities? Would there be a ban in Britain on the use of DDT if it only killed peregrine falcons and did not accumulate in human food chains also? The author argues that most of the justifications of conservation carry little weight with the general public and governments. He demonstrates weaknesses in the ethical, aesthetic, economic and scientific cases, believing that the strongest argument is ecological: we must protect the bird's environment because it is ours too. Unfortunately, many bird species could doubtless be destroyed without any significant effect on our survival or even on our wealth. The only unshakeable argument is the ethical one but its great weakness is that too few people share it.

Halliday's concluding section is a rather generalised summary of active conservation measures appropriate to endangered bird species and a wholly justified criticism of the lack of research on endangered birds. Of course, there is a dual reason for working on common species. Results are usually more certain and those results may help us to understand and solve the problems of rare and endangered birds. But if concentration on the former means neglect of the latter, the balance of research is wrong: international conservation agencies could help to redress that by careful direction of research funding.

Despite errors and omissions resulting from the fact that some information is five years out of date, which is hardly excusable in a book dealing with species whose status may be expected to change from year to year, *Vanishing Birds* is a helpful introduction to the subject, and the author's colour plates and many line drawings appear both accurate and elegant.



Cancer

Illustration taken from *Ronald Searle*, with an introduction by Henning Bock and an essay by Pierre Dehaye (André Deutsch: London, £14.50)—a superbly produced album of work by the graphic satirist.

In *Birds of Man's World* (British Museum (Natural History)/Cornell University Press: London and Ithaca, £5.95) Derek Goodwin has taken a contrasting approach with an account of his own observations of those birds which are able to exploit man-made environments, notably the urban species which are, to most of us, the common birds of the world.

The book begins with a general summary of the ways in which man's modification of his environment have created widespread conditions which benefit a rather small number of species. Thus the deforestation of Europe, much of North America and New Zealand favours grassland species: buildings provide nesting places for cliff dwellers such as swallows, martins and rock doves which were formerly much restricted by lack of suitable sites; and the ranching of domestic livestock has permitted cattle egrets—recently confined to the Old World—to colonise America and Australia. Perhaps surprisingly, it does not discuss our one outstanding domestic example—the colonisation and spread of the little ringed plover, a species which on the Continent breeds normally on shingle bars in rivers. First recorded nesting in Britain in 1938, it has since spread widely through England to Wales and Scotland, using the growing number of gravel pits created by the expanding aggregates industry.

In some ways, this book tells one more about the author than about the birds: he is refreshingly able to find pleasure and fascination in sparrows and pigeons, fully conscious of the fact that the ordered urban world we find so reassuringly safe is to them as wild and hazardous as a rain forest is to us, and that their biology and behaviour is intrinsically every bit as worthy of study as the activity of more exotic species. But because it is based on personal experience, the book provides an irritatingly incomplete study of the subject. A description of the urban birds of Colombo based on a day visit in 1965 leaves one very conscious of this.

A large proportion of the text discusses food sources but does not develop the subject of the impact of common birds on man's activities, nor of man's reactions

In Britain, the inclination is still to kill birds rather than to find alternative, if more costly, solutions. Gulls, for example, exploit man's wastefulness by resorting in great numbers to rubbish tips, especially in coastal towns. When they stay to roost on the reservoirs and to breed on rooftops or in water catchment areas, man's hand is turned against them—usually by means of ill-considered and ineffectual culling programmes. In turn, kind old ladies, conservation bodies, local councils and water authorities become embroiled in acrimonious and time-wasting disputes, which inevitably divert attention from far more important land-use problems.

Derek Goodwin's final chapter looks to the "Future Imperfect". Like Tim Halliday, and no doubt most professional conservationists, he finds little cause for long term optimism.

John Andrews

John Andrews is Head of Conservation Planning at the Royal Society for the Protection of Birds, Sandy, UK.

Conservation addicts

FOUR recently published books reviewed here are concerned wholly or in part with the effect of human activities on the fauna of the world, especially on the larger mammals. All have much to say about the decreasing numbers of some species, and of the measures of conservation taken to preserve what remains.

Dr Brian Bertram spent four years watching lions in the Serengeti Park of Tanzania, and presents the results of his studies in his well illustrated *Pride of Lions* (Dent: London, £6.50). He lived for over half the time in a shed in the bush, getting to know the local lions individually and tracking some of them by putting radio transmitter collars on them after immobilisation with a knock-out shot of paralysing drugs. His book gives a full account of the life-history of lions, enlivened by stories of his experiences and adventures. He adds chapters on the leopard and the cheetah in which he contrasts the solitary life of these species with the social life of lions. He concludes by discussing the evolution of lions, natural selection and genes, and the impact of man on lions in wildlife parks. Apart from a few solecisms, Dr Bertram writes well.

In *Tiger! The Story of the Indian Tiger* (Collins: London, £7.95), Mr Kailash Sankhala deals similarly with the tiger, but also includes much personal material—as he says, the book might be called the autobiography of a tiger addict. His formal training was not in zoology but forestry; after postings to various Forest Divisions of India, and managing the wildlife sanctuaries in some of them, he took charge of Delhi Zoo in 1965 where he remained for five years. Since then he has made a census of the wild tigers of the subcontinent, which resulted in the setting up of over a dozen tiger reserves to conserve the dwindling numbers of tigers and other animals. The first part of the book describing the natural history of the tiger, and the author's adventures in studying it, is the story of an enthusiastic naturalist enjoying the outstanding richness of India's wildlife. The author discusses, from his extensive experience, the tiger's solitary daily life, its environment, prey, co-predators and camp followers, and the other wildlife, and makes a very good job of it.

In the second part of the book Mr Sankhala considers matters such as hunting, circuses and zoos, the near extermination of the tiger and the efforts made to preserve the species. He also makes a lengthy apology for man-eaters and cattle-lifters, and adds some snooty remarks about the exploits of Jim Corbett in killing them. Nevertheless, tigers do sometimes kill and eat people. He becomes really controversial, however, when he talks about zoos and circuses, both of which he condemns as outmoded and no longer justified. When he went to Delhi Zoo he soon saw that its main role was not education but entertainment, with "riding elephants, begging bears, performing otters, smoking chimps and talking parrots . . . and the Children's Zoo a mere toy house". He did away with all this in spite of the unpopularity it brought upon him, and developed the zoo as "a wilderness with minimum signboards and direction indicators". Yet even though rare animals bred so that he was proud of his success and convinced temporarily of "the importance of zoos in conservation",' at other times he felt he had more blood on his hands than the most hardened poacher. He points out that zoos can never play much part in conserving rare species, and the craze for safari parks and zoos is not the answer to saving them. "Now that ecology, environment and the biosphere are all the rage they are suddenly changing their signboards to become 'Ecological Centres' or 'Biological Parks'. The new zoos are designed on an environmental concept", but even with the best designing they come nowhere near this ideal—even the best zoos are travesties of nature that mock their inmates. "The whole idea of bringing a free-living animal into captivity is revolting, and simply because they eat well and reproduce in captivity does not mean that a zoo is a proper environment for them".

Sick baby orang-utan rescued from an animal dealer

Taken from *Back from the Brink*

As someone has said, a zoo is a lot of wild animals kidnapped from their native freedom and locked up for uncomprehending yahoos to gape at in a sordid slum where they pitifully try to fulfill their innate behaviour patterns. The author points out that wildlife films seen by millions on television are far more educational than animals in zoos, and asks "if it is not necessary to bring the Grand Canyon or the Taj Mahal . . . into your city why should there be any justification for netting and snaring animals for educational purposes at a zoo? . . . Except for a few zoos which do a real job of contributing to knowledge their objectives are pure hypocrisy". Anyone who knows anything about the way zoos are run will, if he be honest, heartily agree. The proper place for wild animals is in the wild.

Mr Sankhala, like Dr Bertram, thinks that nature reserves as alternatives to zoos should be run with the minimum of "scientific" management. Mr Guy Mountfort, in *Back from the Brink* (Hutchinson: London, £5.50) tells of the making of some reserves, and of the ways by which several 'endangered' species have been saved from apparently imminent extermination. He is a dedicated supporter of the 'conservation movement' who draws a picture of himself as the super do-gooder, trotting about the globe addressing meetings, button-holing VIPs—he seems to like dropping names—raising money, and intriguing to get official approval for reserves and animal parks. His material is interesting, but his style of writing is that of the establishment and the old boy network—the man in Whitehall knows best.

Mr Christian Zuber, on the other hand, is a cameraman who specialises in wildlife films that are mainly shown by French television, on which he is a public figure. He, too, is an ardent conservationist but no zoologist; he consequently makes some surprising blunders in his statements about animals in *Noah's Ark: Animals in Danger* (Cassell: London, £8.95), but perhaps some of them are due to his translator. His pictures, however, are excellent, informative and interesting, though some of them cover the same subjects as those in *Back from the Brink*. It is strange that conservationists should write books with such good illustrations but such boring texts.

All these authors are much troubled that the wildlife of the world is rapidly decreasing under human depredation. The causes of world decline are many, some are unavoidable but others unnecessary and disgraceful. Foremost is the expanding human population, steadily encroaching on the wilderness to satisfy the hunger for land and what it may produce. The mad rush by Indians to cut down the forests and shoot all the animals when law and

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Essays in Biochemistry

Volume 14

edited by P. N. Campbell and W. N. Aldridge

December 1978, approx. 150pp., £4.20/$8.75 0.12.158114.4

This well established and highly praised series is intended for senior students and lecturers in biochemistry. Each essay presents an overall view of one aspect of the subject, indicating its origin, present status and likely development. It is hoped that research workers will also find the series valuable as a source of information

Contents of Volume 14

A. K. Kenny and A. G. Booth: Microvilli. their ultrastructure, enzymology and molecular organization. **B. T. Pickering:** The neurosecretory neurone a model system for the study of secretion. **J. R. S. Arch and E. A. Newsholme:** The control of the metabolism and the hormonal role of adenosine. Subject index

Neurotransmitter Systems and their Clinical Disorders

edited by N. J. Legg

December 1978/January 1979, X + 240pp., £7.60/$15.75 0.12.443050.3

This book is based on a meeting held by the Department of Neurology at the Royal Postgraduate Medical School, Hammersmith Hospital, London, in 1977, and reviews selected topics in the field, dealing with them from both clinical and experimental viewpoints The range of topics matches that of the field as a whole, from the simple movement disorder of myasthenia gravis, through more complex motor and behavioural defects due to dopaminergic imbalance, to work on psychiatric disorders such as depression and schizophrenia In addition, the autonomic nervous system and its disorders are dealt with in detail.

Manipulation of the Immune Response in Cancer

edited by N. A. Mitchison and M. Landy

December 1978/January 1979, approx. 350pp., £11.50/$23.75 0.12.500250.5

The major concern in cellular immunology in recent years has been to determine the mechanisms of immune regulation. Many of the achievements so far attained could have direct relevance to the control of immune response in cancer This approach however depends on the existence of antigen specific to tumours and it was the discussion and evaluation of this question which prompted the National Cancer Institute, USA to sponsor a meeting at Oxford in the Autumn 1977 This volume represented the outcome of the meeting and follows the style of the previous Brook Lodge Symposia (Academic Press) of which it is the latest in the series

Multivariate Approximation

edited by D. C. Handscomb

December 1978/January 1979, xiv + 354pp., £12.50/$25.75 0.12.323350.X

Although both the theory and the practical applications of univariate approximation have enjoyed good coverage in books, journals and conferences, multivariate approximation has received very little specific mention, despite potentially more varied and more rewarding applications With a view to redressing the balance the London Mathematical Society convened a research symposium at the University of Durham, from July 21 to July 30, 1977, devoted to multivariate approximation This book contains the formal proceedings of the symposium, which brought together many of the best known people currently active in theoretical or practical aspects of multivariate approximation

Tables of the F- and Related Distributions with Algorithms

K. V. Mardia and P. J. Zemroch

January/February 1979, x + 276pp., £13.20/$27.25 0.12.471140.5

The key role of F,t and X^2 — distributions in statistical methodology has naturally led to inclusion of tables of their percentage points in almost every set of statistical tables. Such tables are however nearly always limited only to integral degrees of freedom and to a few percentage points. This book is believed to be the most extensive tabluation of the percentage points of F yet published and is one of the more extensive of t- and X^2 — distributions. It will therefore be of great value to all statistics workers in universities, agriculture and medical research, and industry

Diabetes, Obesity and Hyperlipidemias

edited by G. Crepaldi, P. J. Lefebvre, and G. Alberti

January/February 1979, approx. 400pp., £16.00/$33.25 0.12.197035.3

Diabetes, obesity and hyperlipidemias are topics normally dealt with separately but the Second European Meeting on Metabolism held in Padua in May 1977, encompassed all three with a view to examining their inter-relationships Among the topics discussed at the meeting and reported in this volume were, the effects of insulin and glucagon *in vitro* and *in vivo* on hepatic metabolism and the role of the liver in carbohydrate metabolism, factors regulating adipose tissue cellularity, fat cell differentiation and development of body fat mass, and the most current ideas on lipoprotein metabolism. Most of the papers reported results of recent research and this book therefore will provide much up-to-date information for all those interested in metabolic disorders including those working on diabetes, endocrinologists, epidemiologists and biochemists

The Pharmacology of Immunoregulation

edited by Georges H. Werner and Francois Floc'h

December 1978, xviii + 460pp., £16.50/$34.25 0.12.745650.3

This book contains the proceedings of the Rhône-Poulenc Round Table Conference, held in Paris in December 1977, which brought together scientists involved in fundamental Immunological research and investigators interested in clinical applications from all over the world The aim of the Conference was to confront the present, still largely empirical, trends in the pharmacological modulation of the immune responses with the rapidly increasing and sophisticated knowledge of the natural mechanisms of regulation of these immune responses

Foraminifera

Volume 3

edited by R. H. Hedley and C. G. Adams

January/February 1979, x + 284pp., £17.50/$36.25 0.12.33640.5

This is the third volume in an open-ended series containing review articles on various aspects of foraminiferal research including biogeography, biometrics, cytology, ecology, oceanography, sedimentology, stratigraphy and systematics. The series provides both specialists and general readers with up-to-date surveys of research, in which critical evaluation is combined with clear presentation.

The contributors to this volume, all experts in their fields, discuss recent advances in foraminiferal fine structure, late Cretaceous orbitoidal foraminifera, and the comparative anatomy of shell structure in some larger foraminifera

Electron Spectroscopy

Theory, Techniques and Applications Volume 2

edited by C. R. Brundle and A. D. Baker

January/February 1979, x + 288pp., £17.50/$36.25 0.12.137802.0

This series brings together the diverse types and applications of electron spectroscopy.

Contents

C. S. Fadley: Basic concepts of X-ray photoelectron spectroscopy. **S. T. Manson and D. Dill:** The photoionization of atoms: cross-sections and photoelectron angular distributions **J. T. J. Huang and J. W. Rabalais:** Ultraviolet photoelectron cross-sections and angular distributions. **J. T. J. Huang and J. W. Rabalais:** X-ray photoelectron cross-sections and angular distributions. **G. K. Wertheim:** Final-state structure in XPS. Subject index.

Rotating Fluids in Geophysics

edited by P. H. Roberts and A. M. Soward

December 1978, xviii + 552pp., £17.50/$36.25 0.12.589650.6

The subject of rotating fluids is worth studying for many reasons, not least of which is that all the large scale motions of the Earth's atmosphere, oceans and core are dominated by its rotation This book is devoted primarily to a theoretical exposition of the subject, although several sections are centred on experimental and numerical techniques in their applications to the atmosphere, oceans and core of the earth. A theme running through the book is waves of stability, supported by a brief section expounding hydrodynamic stability theory This book will bring the student and researcher up to date with the latest findings in the field as well with some fresh developments of the basic principles. It will interest not only postgraduates in fluid mechanics, meteorology, oceanography, and geophysics, but the more theoretically inclined astro-physicist or cosmologist.

Advances in Optical and Electron Spectroscopy

Volume 7

edited by R. Barer and V. E. Cosslett

January/February 1979, xii + 398pp., £19.60/$40.50 0.12.029907.0

This series attempts to cover all aspects of microscopy In this volume two articles deal with problems arising from the study of moving objects, where the application of modern technology offers solutions to long standing problems In the remaining articles the emphasis is on the physical principles of image formation and interpretation, such as the determination of the dimensional structure of macromolecules and the prospects for imaging single atoms. As with the previous volumes it is intended that this volume will be of interest to all those who use microscopy in both the biological and physical sciences.

Fundamentals of Freeze Drying

edited by R. D. Mellor

January 1979, xxviii + 386pp., £20.50/$42.40 0.12.490050.X

The search for the best means of preserving perishable foodstuffs has been continuing throughout the history of mankind. Until recently, both freezing and drying have been practised, but have never been combined Only over the last two or three decades has the process of lyophilization or freeze-drying been developed and used extensively The need for a systematic and scientific approach was clearly acute so since 1958, international courses have been run to investigate the physicochemical bases of the process and to monitor its applications This book reflects this concern and sets out in a comprehensive fashion the background to the principles for the use of both research and technical workers as well as students in food science, process and chemical engineering, physics and biology

Virus-transformed Cell Membranes

edited by Claude Nicolau

January/February 1979, viii + 408pp., £21.00/$43.50 0.12.518850.9

The information contained in this book should clarify many aspects of membrane structure and function in virus-transformed cells. Three areas of research in particular have been investigated: the identification of the surface property or properties associated with loss of growth control, the definition of the surface glycoconjugate alterations that are associated with high oncogenic potential, and the definition of surface membrane changes common to cells transformed by viral, chemical or physical agents Also discussed in detail are transport across the membrane of virus-transformed and serum-stimulated cells, and the use of lectins as probes of the transformed cell surface

Selected Topics in Graph Theory

edited by Lowell W. Beineke and Robin J. Wilson

February/March 1979, xii + 452pp., £21.50/$44.50 0.12.086250.6

One of the consequences of the recent rapid expansion in graph theory is that it has become increasingly difficult to ascertain what is currently known about any particular topic in the field In view of this the editors have collected together a series of expository surveys written by a distinguished group of authors, covering various areas of graph theory An important feature of the book is that a uniform terminology, style and notation has been adopted throughout While some knowledge of elementary graph theory is assumed, all the necessary definitions and theorems used in the book, are described in the first chapter thereby making the book most useful for advanced courses and seminars

Luminescence Spectroscopy

edited by M. D. Lumb

December 1978/January 1979, xii + 376pp., £24.00/$49.75 0.12.459550.2

Luminescence spectroscopy has become too large a field for a single volume to adequately cover the whole of it, even at an introductory level This book, however, provides sufficient theoretical and experimental groundwork to give a newcomer to the field a firm basis from which to follow up particular aspects of the subject. It reviews the experimental use of both the luminescence process and magneto-optical effects in organic and inorganic systems, as well as giving a certain amount of theory, necessary for the reader to understand the principles of the spectroscopic method

Pulmonates

Volume 2A: Systematics, Evolution and Ecology

edited by Vera Fretter and John F. Peake

January/February 1979, xii + 540pp., £24.50/$50.75 0.12.267502.9

The Pulmonata are that subclass of the Gastropoda which includes all the non-operculate land snails and slugs, and a few families of freshwater, brackish-water and marine snails and limpets, most of which respire, at least in part, with a "lung" Where *Volmue 1* was based on laboratory work, *Volume 2a* of this study is oriented towards "field" problems, dealing with the systematics, evolution and ecology of pulmonates. *Volume 2b* will concentrate on the economic importance of a particular species, *Achatina fulica* Both parts will not only be of immediate value to malacologists, ecologists, physiologists and naturalists, but will provide, along with *Volume 1*, a lasting reference guide to these fascinating molluscs

Archaeology of Afghanistan

From earliest times to the Timurid Period

edited by F. R. Allchin and Norman Hammond

January 1979, xxiv + 452pp., £30.00/$62.00 0.12.050440.5

This book is the first synthesis of the archaeology of Afghanistan, a region often described as the "Cross-roads of Asia"; a melting-pot of cultures between India, Iran and Central Asia. These three areas were the seats of major civilisations in the prehistoric and historic periods: Afghanistan, landlocked in its mountains and deserts, lay on the margins of all of them, involved politically and culturally with each from time to time. This marginal location relative to any one area was also, paradoxically, central to the three together, so that the culture which developed in Afghanistan over the centuries was neither Indian, nor Persian, nor Central Asian, but a unique amalgam of all three

Nucleation and Growth of Thin Films

B. Lewis and J. C. Anderson

January/February 1979, approx. 400pp., £27.50/$57.00 0.12.446680.X

This book provides a comprehensive survey of the theories of nucleation and the initial stages of growth of thin films The first two chapters are concerned with the adsorption of atoms on surfaces and the forces which keep them there With this as a foundation the processes of nucleation and growth of clusters are considered in detail, using both kinetic and thermodynamic treatments Particular attention is paid to the common ground between apparently diverse treatments such as the classical and atomistic nucleation models and the uniform depletion, lattice and ring models of surface diffusion capture

Annual Reports on NMR Spectroscopy

Volume 8

edited by G. A. Webb

December 1978, x + 380pp., £28.00/$58.00 0.12.505308.8

The major aim of this widely-acclaimed series it to provide timely accounts of those areas of NMR spectroscopy where significant advances are being made In recent years the advent of FT—NMR techniques has shifted the emphasis away from proton NMR towards less receptive nuclei such as ^{13}C and ^{15}N which are now being studied routinely in many laboratories This, and recent progress in the theoretical interpretation of NMR parameters, is reflected in this series which covers NMR applications in all branches of science The present volume contains chapters which should appeal to workers in many branches of chemistry, physical, organic, inorganic, theoretical and biological

Advances in Marine Biology

Volume 15

edited by Sir Frederick S. Russell and Sir Maurice Yonge

January/February 1979, xiv + 560pp., £31.00/$64.25 0.12.026115.4

The book opens with a comprehensive review by C J Corkett and I A. McLaren on the biology of *Pseudocalanus*. This is probably the most abundant copepod in temperate latitudes and as such is the most important link in the food chain leading from the phytoplankton to fish The second article by M. R Reeve deals with the nutrition of ctenophores. These act detrimentally in the food chain for they are voracious predators which may at times decimate the copepod population as well as the young fish themselves This is followed by a short article by F S Russell outlining the many ways in which a study of marine biology may have an important bearing on human affairs The volume ends with two valuable reviews on pollution and plankton

Biology of the Reptilia

Volume 8: Physiology B

edited by Carl Gans and K. A. Gans

January/February 1979, xiv + 786pp., £35.00/$72.50 0.12.274608.2

This widely acclaimed series provides an up-to-date and comprehensive review of the class Reptilia. It is divided into sections on morphology, embryology, physiology, and ecology and behaviour, each volume presenting summaries of the state of knowledge in a particular area written by leading specialists in the field well qualified to evalute the achievements in the area The latest volume deals mainly with the venom gland system, the biochemistry and pharmacology of venoms and the production of antisera, as well as an account of digestion in snakes in view of the close association between the two *Volume 8* has much to interest pharmacologists, toxicologists and biochemists

The Medical Management of the Critically Ill

edited by Gillian C. Hanson and P. L. Wright

January/February 1979, xviii +1052pp., £45.00/$93.25 0.12.323650.9

This book will fill a serious gap in the literature on critical patient care and should be particularly useful to doctors and ancillary staff who devote most the their time to the management of the critically ill. It will also be of value to specialists interested in specific aspects of intensive care and to doctors who rarely encounter critical illness but who want guidance on this subject In addition, many nurses and technicians who work in Intensive Therapy Units will find this volume a source of helpful information

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order broke down at independence was typical. A second cause is the commercial value of wildlife products that leads to ruthless exploitation; apart from whales, there is the trade in turtles and their eggs, the gathering of colonial-nesting sea birds' eggs on a vast scale, the poaching of ivory and rhinoceros horn secretly condoned and participated in by corrupt administrations in Africa, the killing of crocodiles and lizards for the luxury leather trade, the hunting to supply the demands of the fur trade with pelts of all kinds, from vicuña to tiger, leopard and other large cats. But international agreements to protect some species and control the trade in their products are now beginning to have some effect.

The worst threat to wildlife, however, is the development of remote places for the tourist trade. Remote and beautiful places are disfigured with airports and huge hotels to which hordes of tourists are brought on package holidays. The once placid countryside is invaded by speed boats and water skiing, spear fishing, discos, noise, disturbance and destruction of habitat. The traders in eagerly bought souvenirs are ransacking the remotest shores of the tropics for rare sea-shells, despoiling island faunas to provide stuffed baby crocodiles and iguanas, and encouraging the poachers to supply animal skins and carved ivory. Wildlife parks where animals are preserved provide no safeguards—tourists may be valuable money spinners, but they have to be housed, air-strips and roads must be made to get them there, electricity, water and sewage works supplied, catering organised, the animals 'managed' by wardens, and so on until the whole thing is as artificial as a 'safari park' in a developed country; the tourist industry is a vast humbug. Guides take the tourists to photograph the animals—Dr Bertram in his landrover once found he was followed closely by eight minibuses and cars full of tourists, and he gives a photograph of a tourist minibus, with camera snappers, harassing a lioness at close quarters Mr Sankhala also remarks that the snap-shotting tourist is so preoccupied with shutter speeds, lens apertures and focussing that he fails to see anything around him, and adds that a visitor to a reserve should bring nothing with him but a receptive mind and take nothing away but what it receives.

It is inevitable that, in spite of the craze for wildlife, within a hundred years the wilderness will be tamed, the larger wild animals that have not become extinct will be contained within fences, and development will have destroyed the spirit of freedom. The only way to preserve samples of the fauna is to isolate reserves in which no human disturbance is allowed, such as the area between Seronera and Lake Victoria that formerly was a closed reserve free from human footprints or car tracks. But will the developers agree to large parts of their countries being sterilised against their enterprise? The call of money is louder than the voice of the wilderness.

L. Harrison Matthews

L. Harrison-Matthews was Scientific Director of the Zoological Society of London from 1951–66.

Protean nature of conservation

THREE of the four books reviewed here (that of Maurice Burton is the exception) could come under the heading of conservation but their diversity emphasises the protean nature of the term. Definitions are many and various (if ventured upon) but I know none so comprehensive and thoughtful as that of Charles Elton in his Ecology of Invasions, *viz.* "some wise principle of coexistence between man and nature, even if it has to be a modified kind of man and a modified kind of nature".

These three recently published books, among many others, suggest that, at the moment, the lion's share of this modification has fallen to nature while man is panting behind. Richard Williamson's *The Great Yew Forest: The Natural History of Kingley Vale* (Macmillan: London, £5.95) is a fascinating and first-hand account of his struggles, as warden of Kingley Vale National Nature Reserve, to understand and maintain the unique yew forest with its interspersed chalk grasslands; it shows how hard it is to stem the influence of man even in a nature reserve. It is a first-rate, thoughtful naturalist's book and will be enjoyed not only for its vivid observation but for its searching out of natural processes.

In contrast, Richard Perry's *Wildlife in Britain and Ireland* (Croom Helm: London, £7.25), published in association with the World Wildlife Fund, is avowedly a compilation about the history of the bird and mammal fauna of the British Isles since the last Ice Age It gives special attention to the social pressures that have, for example, eliminated wolves and bears and subsequently borne hard on predators generally and, by contrast, allowed the penetration by exotic species such as the muntjac and sika deer from patrician zoological collections or nutria and American mink from commercial drive. There is some evidence of the beginning of a modified kind of man in his attitude to nature in that animals are less regarded as simply subserving his needs or pastimes. Mr Perry's collation of the prehistory of

An Introduction to Animal Behaviour
Third Edition
Aubrey Manning
In this new edition, the main aim has been to keep a broad biological approach to the subject. There has been a considerable amount of rearrangement of the material to take account of new attitudes to behavioural problems. Thus, for example, imprinting is now considered under the discussion of development, and the contents of the hitherto separate chapter on hormones and behaviour, are now considered with those on development and motivation where they are most relevant. Sections on communication and on sociobiology are the most important new additions and serve to introduce students to some of the results and concepts in these active research areas
Cloth £12 Paper £5.95

Organization in Plants
Third Edition
W. M. M. Baron
This is the third edition of a very successful text on plant physiology for both introductory biology and botany courses at universities and in sixth forms. The book has been extensively rewritten to keep abreast of recent discoveries Much more information on the growth and development of plants is included to stress that the survival of the plant as a species may depend on *all* stages of its life cycle.
With illustrations throughout
Paper £6 75

Micro-organisms
Function, Form and Environment
Second Edition
Edited by Lilian E. Hawker and Alan H. Linton
This second edition of a highly acclaimed text has been largely rewritten and re-structured to take account of recent developments in the field. The editors have organized the material around the three main themes of physiology and biochemistry, natural history and classification, and interaction with the environment, to produce a logical and readable text covering the whole field of microbiology
With illustrations throughout
Paper £8.95

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our fauna is valuable, even if the details are not minutely documented. I could detect no serious errors, though it was startling to find (p63) the strawberry tree flourishing at 10,000 feet in the Alps. This, presumably, refers to the alpine bearberry (*Arctostaphylos alpinus*), which was previously put in the genus *Arbutus*. The great value of the book is to give us some measure of the mutability of the things we try to conserve quite apart from the ever-increasing pressure exerted by the numbers and activities of man.

A quite different aspect of conservation is treated by Lawrence Alderson in his book *The Chance to Survive: Rare Breeds in a Changing World* (Cameron and Tayleur/David and Charles: Newton Abbot and London, £7.50)—namely, the urgency of preserving the breeds of horses, cattle and pigs which, having gone out of fashion, are in danger of being permanently lost. I would not have thought it possible that, being a straight zoologist, my interest and enthusiasm could have been aroused by a theme so much on the fringe of my usual thinking. But Mr Alderson writes so well and with such conviction that I found myself carried along and taking in my stride the welter of British Whites, North Ronaldsays and Gloucester Old Spots (shades of Michael Innes!). His main contention is that, nowadays, breeders of livestock are too concerned to concentrate on specialised characteristics which suit the immediate market, especially in the conditions of indoor cosseting. So breeds that are thrifty and can live on marginal land tend to die out. Yet these may be just the ones that will be needed when environmental and economic conditions change. The book is a plea that such breeds should be regarded as valuable gene banks and it points to how much has already been achieved by the enthusiasm of recently formed breed societies and by the formation of the Rare Breeds Survival Trust. Darwin would have found this book a worthy extension of his *Animals and Plants under Domestication*, especially as so many of the principles of genetics, which were a closed book to him, have now been unravelled. Nevertheless, the impression conveyed by this book is that there is still a long road to travel before the complexities of inheritance in our farm animals are fully elucidated.

These three books, therefore, illustrate forcibly what I have referred to above as the protean nature of the concept of conservation: yet all have some common aim—that of preserving faunal diversity. Kingley Vale is a unique ecosystem and it is good to know that its ancient yew forest is safe from despoliation and cared for by a warden who wants to know "what makes it tick". The problems of management of the British vertebrate fauna, involving so many divergent interests from those who practise agricultural monocultures to those who cannot bear to see blood spilled cannot even be approached without an evaluation of its present status and past history, such as Richard Perry has tried to provide in his book. Finally, the stock-taking of farm animal breeds and the plans that are being put into action to deep-freeze semen and ova of vanishing strains, whose qualities may be urgently needed in a cloudy future, must counteract the gene pools that, once vanished, are gone for ever. Thus, we hark back to "a modified kind of man and a modified kind of nature". The main kind of modification of man so far exhibited is a deeper appreciation of and concern for the preservation of diversity and richness of ecosystems, habitats, flora and fauna. This is an aim on which, in this country, the Nature Conservancy Council and its allied research branch, the Institute of Terrestrial Ecology, have always concentrated their energies. It is, and will be, an uphill fight until the pressure of man and his activities is lightened—one hopes by planning in the best sense and not by catastrophe.

The last book of the quartet, *Just like an Animal* (Dent: London, £5.50) by Maurice Burton, is just like Maurice Burton—a concatenation of anecdotes about animal behaviour ranging from that of ants to that of his own, and other people's, pets. The theme is altruistic behaviour in animals and many of the episodes quoted are, as Darwin said about the natural history of the Galapagos Islands, "eminently curious", though they do not add up to such far-reaching conclusions.

H. N. Southern

H N. Southern recently retired as Senior Research Officer of the Animal Ecology Research Group in the Department of Zoology, University of Oxford, UK

For herpetological novices

THE latest of the Collins' *Field Guides*—*A Field Guide to the Reptiles and Amphibians of Britain and Europe* (Collins: London, £4.95) by E. N. Arnold and J. A. Burton—is a perfect gem, a delight to the eye and a pleasure to handle—a fine, solid, book with a well designed and illustrated, waterproof cover. In Europe, west of 36E, there are about 45 species of amphibians and 90 species of reptiles, including five sea turtles, all of which are described and illustrated with the aid fo numerous diagrams, 126 distribution maps, and 40 superb coloured illustrations from drawings by D. W. Ovenden. It really does show how dreadfully the British fauna must have suffered from extinctions during the Ice Age—I can hardly wait for our next holiday in Europe.

The book contains some delightful touches of realism which must surely appeal to professional zoologists as much as to absolute novices. For instance, the following approach to identification is recommended—first, "try to match your animal's appearance as closely as possible" to one of the species shown in the plates. Then "check with the text and maps that you are within the range of the species concerned". "If the range fits, consult with the description in the text and, lastly, if all else fails, try using the keys". No hypocrisy here about using keys first, and then checking the descriptions and illustrations.

It is suggested that a photographic record be made of reptiles and amphibians seen, but the keeping of pets is discouraged. "The average semi-moribund captive tortoise bears no comparison with the alert, vigorous, herb-scented beasts that plough through the undergrowth of southern Europe. Observing wild animals is far more entertaining, and less demanding, than the drudgery of keeping them". All interesting animals found dead can, however, be preserved, and the reader is informed that, in emergencies, quite good results may be obtained with "the stronger grades of brandy, gin, anis, slivovica, tuica, rakija, schnapps, or ouzo". Yet another advertisement for a continental holiday.

The World of Amphibians and Reptiles (Sampson Low: Maidenhead, UK, £3.95) is a second attractive volume for people with herpetological interests. Although translated from an Italian original it is quite well written. Indeed, it seems both surprising and unfortunate that the author's name should have been withheld. When reading a book, I prefer to know whose ideas I am absorbing. And although, presumably, written mainly for students and amateurs, the book under review does propound some genuinely interesting ideas as well as others that seem rather odd. For instance, amphibians can tolerate cold better than reptiles, especially in regions where the climate is dry: many Hynobiidae are to be found in the cold, but muddy, tundra regions on the Siberian border. The Plethodontidae "have a fixed mandible and in order to chew or swallow they have to move their jaws, and with them the entire cranium"(?). Nocturnal Bufonidae move with small, clumsy hops, as their hind legs are very short—the causal relationship, if any, is not, however, elaborated.

Interesting comments and speculations are made as to the functions of cloacal and muchal-dorsal glands in snakes, predation by land tortoises, the pineal eye of *Sphenodon* and lizards, egg-teeth and caruncles, the sticky membrane of some chameleon eggs which gives them a hold on a branch, and stomach stones

E. Scholtyseck

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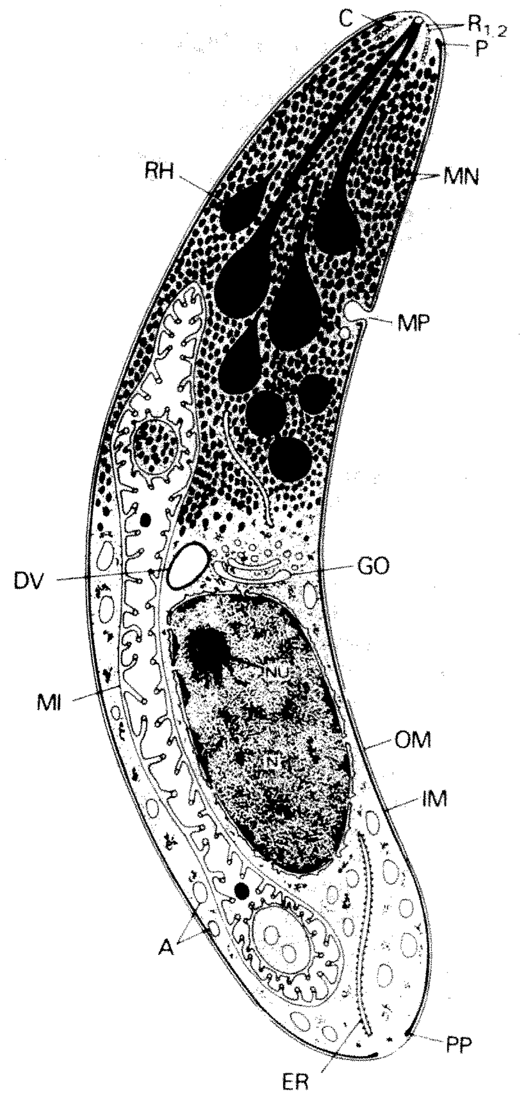

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of crocodilians. We are also told that snakes "spend most of their time half-asleep, and the only stimuli that can rouse them from their state of torpor are hunger-pangs (due to a previous period of fasting, due in turn to laziness) and sex".

On the other hand, there is no serious discussion of the physiological significance of cutaneous respiration in Amphibia; the origin of the cleidoic egg is barely mentioned; adhesion of the gecko's foot to smooth surfaces is explained without reference to friction; the ability of *Chrysopelea* to glide is stated without comment; and physiological thermoregulation in reptiles barely mentioned. The problem of the coloration of coral snakes is not analysed and no reference is made to Mertensian mimicry in this context. Nor can I accept the argument that, as the colours of chameleons are most cryptic when the animals are relaxed, their function is not so much for defensive reasons, but rather to give them a chance to attack a prey unseen.

The two sections into which the book is divided are provided with introductions which explain clearly how each class has evolved to its present status. These sections are followed by accounts of structure, physiology, reproduction, behaviour, ecology, and classification. A detailed bibliography of relevant books is included. The text of some 60,000 words is illustrated by over 200 colour photographs and drawings, but the latter are not always related to the text and are of inferior quality to those of the Collins' *Field Guide*: some are distinctly crude and inadequately labelled. The index too is incomplete. Despite such comparatively minor inadequacies, the book provides an excellent introduction to amphibians and reptiles and can be confidently recommended as a Christmas present that will give interest and pleasure.

J. L. Cloudsley-Thompson

J. L. Cloudsley-Thompson is Professor of Zoology at Birkbeck College, University of London, UK.

Portraits of the Lepidoptera

"KILL not the moth nor butterfly" wrote Blake, and three recently published books reviewed here echo his sentiments, though they are in defence of conservation (or just sheer artistry) rather than because "the Last Judgment draweth nigh". All three have superb illustrations and none refers to the killing bottle or setting board.

Robert Goodden's *British Butterflies: A Field Guide* (David and Charles: Newton Abbot and London, £4.50) is perhaps the best organised. It really is a field guide, and starts with a section on the life-cycle, breeding and conservation of British butterflies, and one can see from it how much there is still to be learnt about their habitats. Every species is photographed alive and with each there is a rough distribution map. For the beginner difficulties of size are overcome by the charts at the front and back of the book, which show the insects (the common ones at the front and the rarer ones at the back) at approximately three-quarters their size. There is advice on how to encourage butterflies to visit gardens and an exhortation for the enthusiast to join the British Butterfly Conservation Society in Leicestershire. The book is for the uninitiated of all ages, and for the old hand is a "must" simply for the breathtaking photographs.

Umberto Parenti, in *The World of Butterflies and Moths: Their Life-Cycle, Habits and Ecology* (Orbis: London, £5.95) looks at some of the many facets of the world of butterflies and moths. The photographs are of unbelievable beauty, and it seems churlish to have to criticise them by saying that they are arranged often randomly, both geographically and for the text, and that there is no indication of the actual size of the insects. The book is at its best in the information which it gives about the evolution of the Order, its anatomy and physiology, the methods of reproduction and the way the insects adapt to their environments, with sections on mimicry, camouflage and pheromones. The least satisfactory section is the classification, which is difficult to take in, and being necessarily short is over-compressed for such a vast subject. In spite of some unevenness the book is strongly recommended, and would make a splendid Christmas present.

Stuart McNeill's examples of British butterflies and moths, in *Butterflies and Moths* (Michael Joseph: London, £11.95) have been selected from the famous series of Christian Sepp and his son, the engravings first being published in Holland in 1762. The introduction explains the technique of copper engraving and gives a history of the Sepp family. The text relating to the insects is up to date, and one notes that the Peppered Moth was true to its name in the Sepps' day—the "dark, Satanic mills" which were to bring industrial melanism being still in the future. The book is recommended more for bibliophiles and nature lovers than for either beginners or specialists in the Lepidoptera. The restrained beauty of the plates makes the exuberance of the colour photographs in the other books seem vulgar, and yet it is interesting how accurately both can portray the insects.

Cyril A. Clarke

Sir Cyril Clarke is Honorary Nuffield Research Fellow in the Department of Genetics, University of Liverpool, UK.

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Natural selection in wildflower books

WILDFLOWER BOOKS in Britain are now proliferating at a rate which almost matches that of bird books. This is indicative of a large and growing market among adult amateur naturalists as well as among the more enthusiastic element within school and university classes. It is not easy to classify flower books, but one might begin by dividing them into field guides, the chief intention of which is to aid the process of identification, and coffee-table books, which rely on the aesthetic appeal of elaborate photography or artwork to attract a market. On thumbing through a selection of recently published flower books one soon realises that this dichotomy is unreal and that most books actually lie along a continuum of variation between these two extremes.

In Britain we have been well supplied with illustrated field guides, in particular D. McClintock and R. S. R. Fitter's *Pocket Guide to Wild Flowers* (Collins: London, 1956) and W. Keble Martin's *Concise British Flora in Colour* (Ebury: London, 1965). Both of these sought to provide comprehensive coverage of the British flora in the form of illustrations and notes on identification and distribution. Many taxonomic botanists have been unhappy with the development of such books, for students are apt to use them uncritically when identifying plants and prefer to look through pictures rather than work through keys. Undoubtedly such misuse of these books does occur, but on the other hand illustrations can be most valuable in confirming identifications and in exciting the interest of incipient naturalists. On this ground alone, in my opinion, they justify their existence.

In a similar vein to these earlier works is *Wild Flowers: The Wild Flowers of Britain and Northern Europe* (Collins: London, £5.95) by M. Blamey, R. Fitter and A. Fitter, which is now available in a large format, hardback edition having previously been published as a paperback pocket-sized book. Illustrations are now, unfortunately, separated from the text and although a taxonomic order is adopted, only English names appear on the plates. This is a great disadvantage over the Keble Martin text, especially where students are concerned. Some of the English names used are not entirely satisfactory, such as 'dwarf thistle' for *Cirsium acaule*, instead of the usual 'stemless thistle'. Some habitat details are inaccurate: *Dianthus superbus* is said to be found in dry places; in Fennoscandia it is found largely on riversides. Problems have arisen as a result of the separation of illustrations from the text (they were interleaved in the paperback edition); there are errors in cross-referencing and those species which were mentioned but not illustrated in the paperback are now omitted entirely. This means that the book is far less comprehensive (such a common species as *Cirsium palustre* is lacking) in its coverage.

The justification for this new format edition with reduced text is the desire to display Marjorie Blamey's illustrations to better effect. This is certainly achieved and the plates are of a high quality. One must, however, compare them with those of Keble Martin and they fail to measure up to the earlier paintings on either aesthetic or technical grounds. Take, for example, the pictures of *Eriocaulon septangulare*; in Keble Martin's illustration the flower structure is clearer, the grooved, spiral stem is visible and the cell structure of the leaves is evident. All of these are useful criteria for identification and are lacking in Marjorie Blamey's work. It is only fair to add, however, that recent editions of Keble Martin's drawings have deteriorated in their quality of reproduction.

Roger Phillips' *Wild Flowers of Britain* (Pan: London, paperback £4.50) approaches the illustration problem by means of a series of colour photographs, yet it remains essentially at the 'field guide' end of the spectrum. The approach has been used quite successfully in the past by Oleg Polunin in such books as his *Flowers of Europe* (Oxford University Press: Oxford, 1969). There are two novel features in Phillips' book: the plant material in the photographs is picked and arranged in a studio setting. The photographs are most effective and the freshness of the specimens is extraordinary. One could object to the technique on conservation grounds but rarer species such as *Gentiana verna* and *Dianthus gratianopolitanus*, are either represented by garden specimens, or habitat shots are used which do not necessitate the destruction of the plant. The other novel feature of the book is the arrangement, which is based upon flowering time, and where appropriate it includes fruit in due season. The coverage is quite full, including some non-native species such as *Cornus mas*, and the mixing of illustrations with text makes it easy to use. This is a valuable contribution to the popular botanical literature, though I still prefer the drawings of Keble Martin.

The Hamlyn attempt to invade the field guide market with Helen Pursey's *Wild Flowers* (Feltham, UK, £1.50) falls short in every respect except its low price. Only 220 species are dealt with and yet the geographical coverage includes northern Europe. The selection of plants included seems to be random; for example, the only species of *Melampyrum* described is *M. cristatum*, which is probably the least likely species of the genus to be encountered in northern Europe. On these grounds alone it is more likely to frustrate than to satisfy or inform the beginner.

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Collins (London, £5.95) has recently taken the daring step of issuing *An Atlas of Wildflowers of Great Britain and Northern Europe* compiled by Alastair Fitter, consisting of almost 2,000 maps of species distribution in northwest Europe. It is a daring step in that such a book cannot hope to have the immediate appeal of the colour picture book, yet it must be regarded as one of the most valuable contributions to popular botany of the decade. Apart from the obvious use of such maps in plant hunting, the enthusiast who has learned basic plant identification from the field guides will now be able to consider what environmental factors, such as climate and human modification of habitats, are instrumental in the production of the distributions described here. It should also provide a more balanced attitude to conservation of the British flora, for, although such glamorous species as *Cypripedium calceolus* are bound to attract much effort on the part of preservationists, some of the most distinctively '*British*' plants are the oceanic heathland types such as *Ulex gallii*. This realisation may increase public awareness of the need for the conservation of these internationally scarce habitats which we rather take for granted.

Lys de Bray's *The Wild Garden: An Illustrated Guide to Weeds* (Weidenfeld and Nicolson: London, £6.95) concentrates on weed species and falls closer to the coffee-table end of the spectrum than the field guide end. It is very much a folk lore type of book with an abundance of legendary material and herbal recipes. John and Susan Proctor's *Nature's Use of Colour in Plants and Their Flowers* (Peter Lowe/Eurobook: London, £3.95) also gives an initial coffee-table impression, especially in its lavish full-page colour illustrations of flowers like *Strelitzia* and of fruits like the apple. It does, however, go far beyond this level in discussing the nature and function of plant pigments, and of pollination mechanisms. A chapter on plant mimicry is particularly valuable, as this is a subject rarely dealt with in popular texts. Both Batesian and Mullerian mimicry are explained and illustrated from the plant kingdom. Parts of this book could be read by sixth-form (advanced school) students and first-year undergraduates with great benefit.

North American plant books are represented in this list by a field guide, *Wildflowers and Weeds*, by B. Courtnay and J. Zimmerman (Van Nostrand Reinhold: Wokingham, UK, paperback £5.30) and by a coffee-table book, *Wild Flowers of Canada*, by M. Ferguson and R. Saunders (Van Nostrand Reinhold, £15.15). The latter contains some superb colour photographs of plants arranged in habitats and links these to brief, if prosaic, text descriptions. With only 150 species described it does not provide adequate information for identification. *Wildflowers and Weeds* consists of a series of small colour photographs covering 650 species from the Great Lakes area. Arrangement is taxonomic and text notes are reduced to a bare minimum. The small size of the photographs and their poor quality of reproduction limit the value of this book, which otherwise emulates the approach of Polunin in Europe. It presents no challenge to the Tory Peterson guides in North America.

Finally, there is a coffee-table book by W. P. A. Jackson, *Wild Flowers of Table Mountain* (Howard Timmins: Cape Town, £12.95), which has some excellent photographs of flowers growing in their natural setting on Table Mountain, South Africa. The text is informative and interesting and should appeal to horticulturalists with a penchant for the Cape flora.

The radiative evolution of flower books seem to be operating on a number of major lines. Photographs are taking over from detailed and accurate artwork, which is unfortunate. Some attempt is being made to provide information over and above that which is relevant to identification; this is to be welcomed. What needs to be developed is the use of simple keys to add precision to the identification processes (for example, with the yellow Composites) and the placing of plant species into habitat contexts so that their interactions can be better appreciated.

Peter D. Moore

Peter D. Moore is Senior Lecturer in the Department of Plant Sciences, King's College, University of London, UK.

Orchid guide

A Field Guide to the Orchids of Britain and Europe with North Africa and the Middle East. By J. G. Williams, A. E. Williams and N. Arlott. Pp.176. (Collins: London, 1978.) £4.95

SOONER or later, most amateur botanists reach a point the popular, general floras seem not to describe their latest discovery, while the specialised works are too bulky, too costly, or only available in a library. Those whose problem lies with identifying wild orchids, not only in Britain, but virtually anywhere within reach of the home-based holiday traveller, should be frustrated no longer, for this new field guide ranges geographically from the Azores to the Urals, by way of Iceland and the countries of the Maghreb. It describes and illustrates 138 species, as well as a large number of sub-species and varieties, is small enough to go in the pocket, and is very reasonably priced.

Oncidium lanceanum (Orchidaceae)

Mary Evans Picture Library

The lay-out is convenient, each description being faced by the relevant illustration, although the very small type may prove a problem for those whose sight is not what it was. A few introductory paragraphs are followed by a key to the genera—which seems a trifle unnecessary—and more detailed keys to *Ophrys* and *Orchis*; the authors have wisely not attempted a key to the complex genus *Dactylorhiza*. In general, the *Flora Europea* classification has been followed, but it is unfortunate that means of correlating this with the nomenclature adopted in older floras are not provided. Descriptions of the individual species are in general sufficient and accurate, although there may still be some frustration over sorting out the scarcer Mediterranean species of *Ophrys*, and, inevitably, the Dactylorhizas. Some mention might have been made, too, of the many interesting, puzzling and not uncommon inter-generic hybrids which are features of the family as a whole. One might also query some of the authors' assessments of the rarity of certain species, and their data on distribution are not always as complete or up-to-date as they might be.

The illustrations are perhaps somewhat less satisfactory. At least as here reproduced, they do not reach the high standard attained in other books in this series, and it is at times evident that, as is made clear in the Introduction, the artist has worked from the authors' sketches, and has never seen the living plant. Considerable attention is given, too, to enlargements of parts of no particular value as aids to identification by the ordinary amateur. Two excellent features that follow the main text are an unusually full glossary, and a check list wherein the reader can record his or her own personal finds. In general, these are minor criticisms of points easily rectifiable in a later edition; they certainly do not detract from the general usefulness of a book, which fills a notable gap in the amateur botanist's library. **Peter Collins**

Peter Collins is a freelance science writer who has been studying the orchids of Europe for more than 25 years.

Armchair geology

THE mere statistics of wilderness areas are impressive enough. The Earth's surface contains something like 18×10^6 km^2 of desert, about half of which is accounted for by Australia and the Sahara. And to this 12% of the total land surface must be added 11% under permanent ice cover, 9% covered with mountain, a further 9% of cold and waterlogged tundra, and 29% of otherwise uncultivable continent comprising semi-arid regions, bare rock, frost-prone areas, and so on. Taking the oceans into account now, only 9% of the Earth's surface can be cultivated and only about a third of that is actually tilled.

To be able (by courtesy of the BBC) to escape from man's narrow well-beaten tracks from time to time to make a series of television programmes about the world's varied wildernesses, Anthony Smith, the naturalist-broadcaster (as opposed to the political scientist-broadcaster and the medical journalist of the same name), was indeed privileged. His book, *Wilderness* (Allen and Unwin: Hemel Hempstead, UK, £7.50), though obviously a spin-off from the series, is not the "book of the film" in the usual sense but, rather, an independent personal account involving a mixture of description, observation, reaction, interpretation, background and anecdote relating to his visits to the world's largest ice-sheet (Antarctica), the biggest tropical forest (Brazil), a large desert (central Australia), the highest mountain range (Himalayas), the tundra (northern Canada) and the most extensive swamp (Sudd).

"Discovering a nest", says Smith at one stage with unconscious irony, "is more rewarding than being shown ten of them." Precisely—which is why all travel books are inherently unsatisfactory. Whether you like or dislike this one will depend on whether or not you can bear to visit places through another person's words. Generally I cannot; but because of the unusual nature of the places, I managed to overcome the resistance in this case and was rewarded with a pleasant afternoon, not least because of the superb colour plates.

But if Smith's book is the closest most of us are likely to get to the true wilderness, the phenomena described by P. J. Banyard in his *Natural Wonders of the World* (Orbis: London, £5.95) are more accessible, as they lie mainly within man's proportionally small terrestrial domain. Landforms, ice patterns, water displays, atmospheric phenomena, gurglings from the Earth's interior, exotic flower arrangements—they are all here in a combination of pleasing pictures and straightforwardly factual text. It makes for a nice browse; but there is a sense of *deja vu* about it all, for what we are looking at is just the latest version of the world picture book that grandma bought us for our eleventh birthdays. There is little one can fault Banyard on; lest it be lack of inspiration; but it is at times like this that one comes to appreciate Anthony Smith's sense of personal quest and involvement.

Involvement of another kind is inherent in Alan Woolley's *Illustrated Encyclopaedia of the Mineral Kingdom*, (Hamlyn: Feltham, UK, £5.95) for mineralogy in its simplest form, the collection of minerals, is the most popular of geology's branches, at least as far as the amateur is concerned. I presume that this new Hamlyn encyclopaedia has been produced largely with the non-geologist in mind, but non-mineralogical Earth scientists will also find it useful as far as it goes. Its main defect as far as working geologists are concerned is that it only goes as far as about 300 of the world's 2500 or so minerals (generally the most common and widely distributed but including a few rarer ones of greater than usual interest to mineral collectors).

For the chosen 300, however, all the essential information is given (composition, hardness, lustre, occurrence, and so on) and the colour photographs are good. On the other hand, the book is far from being a mere list, for there are summary chapters on the Earth's structure and history, crustal composition, minerals and rocks in their geological environments, crystals, gemstones, economic minerals and the building of a mineral collection. This book too is but a variant of its numerous predecessors over the years; but within its stated limitations it is far more authoritative and better produced than most.

Aggregates of minerals (that is, rocks) are generally less colourful than some of the pure minerals and thus less popular as collectors' items. The most colourful specimens in *Rocks of the World*, (Pergamon: Oxford, £15), which is not a book but a neatly-mounted set of 36 of the world's most basic rocks, are the laterite, the serpentinite and the ignimbrite; but despite the preponderance of greys the collection is a fascinating one, not least by the way it suddenly appears in one's hand without all the effort of collecting individual samples. Armchair geologists of the world will rejoice; but schoolteachers will not be displeased either, for a good collection of basic rocks is, of course, essential for beginning geology classes. The samples are rather small (up to about 4 cm across) and, being mounted, cannot easily be handled by a group of students. As a purely visual display, however, the set can hardly be faulted.

Peter J. Smith

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For budding holographers

Understanding Holography. By Michael Wenyon. Pp. 176. (David and Charles: Newton Abbot and London, 1978.) £5.50.

THIS book is intended for the general reader and starts with two chapters about light and the laser in which various ideas relating to waves, coherence, interference, stereoscopic images, stimulated and spontaneous emission, and so on, are introduced in a non-mathematical manner. There follows a short digression on the applications of lasers in such fields as medicine and laser light shows before a discussion of the principles of holography. The basic ideas of holography are considered from the diffraction grating approach, and a well informed reader may have thought that more insight into the process would have been obtained, had the idea of the grating been extended to include the Fresnel zone plate. The effects of the size and spectral content of the light source used in the reconstruction process are, surprisingly, not considered in detail.

The chapter on display holography makes entertaining reading and there are many illustrations (necessarily two dimensional) of what has been achieved in this field. It is possible to make holographic pendants, three dimensional abstract light patterns and rainbow cylinder holograms; and even Salvador Dali has shown an interest in the technique. As for the future of holography, the author tells us that holographic movies already exist and could well become a viable proposition on a large scale. There is, however, a sinister comment that "life-like 3D movies could also be used in the future for psychological conditioning or brainwashing". Holographic television, it seems, is unlikely to be realised in the near future Apart from its obvious entertainment value, holography can be used in a more serious vein, as illustrated by the accounts of applications in the study of the vibration modes of musical instruments and the testing of car tyres.

The final chapter is an account of how to make your own holograms with simple equipment. Perhaps the easiest way of making a simple hologram is to use conventional fine grain film and to ensure there is a small angle between the object and the reference beam. It is a pity there is no mention of this experiment, if for no other reason than the relative cheapness of the photographic material. Also the double exposure scatter plate experiments could have been described as they are easy

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This book comprises a large collection of papers written by the most eminent workers in the field of prostaglandin research. The book contains most of the material presented by the authors at a symposium on the "Chemistry and Biochemistry of Prostanoids", held at the University of Salford, England, in July 1978, and charts the progress and direction of prostaglandin research in the past eighteen months.

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to do with conventional photographic film. The final sentence is a warning never to look directly into a laser beam. Perhaps there should also have been a suggestion that the budding holographer ought to consult an expert before he purchases any equipment. It is so easy to labour in vain.

A. Thetford

A. Thetford is Lecturer in the Section of Applied and Modern Optics in the Department of Physics at the University of Reading, UK.

Astronomy for the amateur

ASTRONOMY is a subject which lends itself to the lavish illustrator. On the one hand you have the photographs, a plethora of nebulae, galaxies, star fields, planetary surfaces and lunar landscapes; on the other, you have the imaginative artist only too keen to paint what the eye cannot see (or being ever the optimist I should say "hasn't yet seen"). And with figures, scale diagrams of satellite and planetary systems, galactic clusters, binaries, stellar cross-sections, geological and planetological features, the list could be enormous. I'm a great believer in the adage that a good picture is worth a thousand words and it is obvious that many authors of astronomy texts are too. Looking through some old and some not so old books for the astronomically minded layman, one obvious change with time is the ratio between diagrams and text. In the three astronomical 'glossies' I have before me now this ratio seems to be way above 70%. And why not? What an enchanting feast for the eye they are. The best is by Peter Ryan and Ludek Pesek (*Solar System*, Allen Lane: London, £9.95). Ryan's text gives an easily understood accurate and up - to - date account of our knowledge of the Sun, the planets and their satellites. Pesek's paintings are absolutely fascinating, magical insights into Venusian clouds, Saturnian rings, Jovian electrical storms and a host of planetary and satellite landscapes, all with a starkness that is breathtaking. The photographs are well chosen and beautifully reproduced. But special praise must go to the studio (QED) who produced the diagrams; these are superb. This book is an enchanting guide to the interplanetary age.

Dropping the price by a factor of three we have Ian Ridpath's *Stars and Planets* (Hamlyn: Feltham, UK, £2.95), a lavishly illustrated, rather thin, romp through the history of astronomy, the Solar System and the Universe. On a price per page basis, it is comparable to Ryan and Pesek, but I like Ridpath's style. It bubbles along infectiously and makes this book an ideal introduction to the wonders, beauties and new things in astronomy.

My final big book is a revised edition of the *Mitchell Beazley–Patrick Moore Atlas of the Universe* published in 1970 (*The Mitchell Beazley New Concise Atlas of the Universe*; Mitchell Beazley: London, £12.95). The blurb stresses the fact that it is "new" and "completely revised", but on reading the book one gets an impression rather like opening a packet of "new" "improved" soap powder. The contents differ little from the previous. Not of course that this is altogether a bad thing. The 1970 edition was very good, and these good features remain in this new edition: the star maps, the moon maps and the potted descriptions of a host of astronomical objects. The planetary sections have obviously changed the most and they contain results from all the recent space probes. The book is an excellent aid to learning, ideal for the new astronomer and the schoolroom. If you haven't got the first edition then I recommend this book heartily. If you have the first edition, stick to it and save your money.

Staying with the younger reader for a while we have *Astronomy: An Introduction* by Jacqueline Mitton (Faber: London, hardback £6.25; paperback £2.75). This small format book has about 120 pages of text and its audience seems to be scholars taking the Ordinary Level (middle school) Astronomy examination. Its main usefulness seems to be in what it leaves out. Here is what you would need to know to pass the exam—no more, no less—so it is ideal for a busy scholar but is rather skeletal for others.

The plaintive knockings of Walter de la Mare's horseman are echoed in my next two books. As the Earth-bound traveller, or should I say a few of our travellers, call out to the Universe, "Is there anybody there?", Iain Nicolson (*The Road to the Stars*, David and Charles: Newton Abbot, UK, £5.95) encourages us to build spacecraft and go and see, whereas Ian Ridpath (*Messages from the Stars*, Fontana/Collins: London, paperback 95p) is more content to stay put and broadcast our presence and to listen intently for answers or other communications from "out there". Ridpath starts his book by inquiring if life exists elsewhere in space and informing us that "This is the single most important scientific question which we are currently capable of answering, and certainly the most exciting." Well is it? I'm not convinced. It would probably take much more than Little Green Men in Trafalgar Square to shake us from our xenophobic proclivities, and the importance and the excitement of the possibility of there being other peoples than ourselves doesn't seem to be shared by those world scientists intent on the conquest of disease, poverty, famine and war on a planet whose resources seem to be shrinking as rapidly as its population is exploding.

Ridpath discusses in layman terms the factors that go into the Drake formula (the formula that gives the number of advanced civilisations which exist at the moment and which have the capability for interstellar communication) and the attempts that scientists have already made to find extraterrestrial life, both by experimentation on Mars and by using radio telescopes to listen out. The book considers the humanisation of space and the concepts of space colonies, the message we expect others to send to us, how we would communicate, the consequences of contact, and the possibility that we have already been visited. I found the book a most enjoyable read and a commendable starting point in this engaging subject.

Nicolson's approach is less partisan than Ridpath's. He seems to aim more at the dedicated amateur astronomer than the "man in the street". It is a book which, with exemplary clarity of presentation, brings the reader to and often beyond the frontier between space science and science fiction. Nicolson is discussing star travel, the human race colonising the galaxy. He answers such questions as what spaceships will we use?, "how will they be propelled?", "how long will it take us?", "is it worth it?", and "when will we be ready to go?" Section headings such as "The consequences of contact", "Relativistic starships", "Suspended animation—The practicalities and prospects", "Spinning black holes", "The laser photon sail", "Time dilation", and "The roads to the planets", reveal some of the many feasts that lie in store for the reader. This is an eminently sensible book in a field which is often a happy breeding ground for the over-imaginative. It is a clear, considered scientific exposition of the problems and possibilities of stepping out into the reaches of space. I was impressed. But still the nagging possibility remains that there is no-one out there—no aliens to visit, no reasons to go. What happens if after shouting out our presence, returning to the words of de la Mare, "the silence surged softly backward". I think that realising we are alone would be as disturbing as realising we are not.

Finally we have two books for the telescope owners. I need say very little about Jack Newton's book, *Deep Sky Objects: A Photographic Guide for the Amateur* (Gall Publications: Toronto, hardback £5; paperback £3), other than if you enjoy looking through a telescope, get it immediately. It is a superb practical guide for finding the Messier objects and some of the more interesting of the NGC objects. It contains clear finder charts for all of them and also photographs of each object taken with the author's 12.5-inch Newtonian telescope. Not only are you told where and when to look you are shown what to expect. All astronomers should say a big thank you to Jack Newton for making their telescope time more productive and more enjoyable.

Using the Telescope, by J. Hedley Robinson (David and Charles: Newton Abbot, UK, £4.95) is a different matter. I will agree with the author that a book in which the amateur observers could find "comprehensive instructions for the proper care and maintenance of their telescopes, together with explicit explanations of correct observational techniques for all celestial bodies that they are likely to be interested in" would be extremely useful. Unfortunately the book under review isn't it. Robinson doesn't go far enough. The budding astrophotographer is told next to nothing about the science of photography and is fobbed off with the 'trial and error method'. The section on telescope mounting is complicated by a lack of diagrams and by mislabelling the two that are given. When it comes to observational details our appetites are wetted and then we are left up in the air. I'll give you a typical example: "Since then many TLPs have been recorded in a number of craters, both inside and out"—we aren't told which craters. The spectroscope "is equipped with one or more prisms, set at suitable angles for the light to pass through each in succession," but we are given no hint as to what the angles might be or what prisms to use. I think the amateur observer would do much better by saving a bit more money and buying *Astronomy: A Handbook*, edited by G. R. Roth (Springer: Berlin, 1975).

David W. Hughes

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Portrait of five heroes

This Wild Abyss: The Story of the Men Who Made Modern Astronomy. By G. E. Christianson. Pp. 461. (Free Press/Macmillan: New York; Collier Macmillan: West Drayton, UK, 1978.) £9.75.

WHAT a pity! as Sir George Sitwell was fond of remarking: such a wasted opportunity. It is a long time since a general book on the history of astronomy was published and it will be some years yet before the new scholarly history (sponsored by the IAU), now in preparation, is before us. Although one's initial expectation that the "makers of modern astronomy" might include such figures as Hubble and Shapley is proved mistaken, a sound survey of the history of astronomy to Newton would be worth having as so much new work has appeared in recent years. There has been the debate about megalithic observatories, many exciting discoveries made about late Islamic astronomy by E. S. Kennedy and others and the outpourings associated with the anniversaries of Copernicus and Kepler, not to mention whole new worlds of scholarship devoted to Galileo and Newton. A new survey of early astronomy could be both more technically competent and more rich in content, as well as more vivid in its discussions of problems, than anything that has appeared yet. Mr Christianson, however, has only had a slight success in releasing himself from a familiar and by now dated pattern.

He makes no claim to be a technical historian of astronomy, which is fair enough; his chief aim has been to write biographies of five great figures: Copernicus, Brahe, Kepler, Galileo and Newton. But he writes, as perhaps he could not but do, about a good deal else including Greek astronomy and medieval civilisation. A general history (even by a non-specialist author) should get the broad historiography and the basic facts right. Mr Christianson tells us that Ptolemy's orbital elements "did not fit comfortably into" the crystalline spheres of Aristotle "and whether or not Ptolemy even believed in such spheres is not known; they receive no mention in his writings". Not so; solid spheres figure prominently in Ptolemy's *Planetary Hypotheses* and are indeed mentioned occasionally in the *Almagest* itself (see O. Pedersen, *Survey of the Almagest*, 1974, 167, 391–7).

Mr Christianson's whole discussion of ancient astronomy is impaired by his failure to grasp the point emphasised seventy years ago by Pierre Duhem that the Greek astronomer was a mathematician, not a philosopher; and correspondingly that it is impossible to appraise the Copernican revolution properly without first seeing the significance of his opposite position that mathematics, far from merely saving the appearances, is an essential key to physical reality. Mr Christianson seems unfortunately to be wholly unaware of the writings of Duhem—widely regarded as providing the basis for the historiography of medieval science as it has flourished during this century—and only ignorance, again, can account for his extraordinary statement that "the study of the Middle Ages" has "until rather recently languished in our institutions of higher education".

Although the author is more familiar with recent studies of Galileo and Newton, his biographies are still marred by serious mistakes, such as the assertion that Galileo discovered the law of falling bodies by rolling "objects of different weight . . . down an inclined plane . . . keeping time with a specially designed pendulum"; his belief that Descartes proposed to correct *chromatic* aberration by means of aspherical lenses; and his supposition that Newton plunged deeply into the study of the "occult" (Newton did indeed read deeply in alchemical authors and interpret scriptural prophecy, but no-one has yet made him out to be a warlock).

In general, the ideas of established standard works like T. S. Kuhn's *Copernican Revolution* and Giorgio de Santillana's *Crime of Galileo* are fairly represented, and with Newton the author has made use of Frank Manuel's psycho-analytical portrait, Mrs Dobbs's investigation of Newton's alchemy, the publications of R. S. Westfall, and other recent sources. He ends with an exposition, highly laudatory, of Kuhn's *Structure of Scientific Revolutions* with which, he writes, he agrees that "there is nothing absolute in what is called scientific truth", that is, a statement is true so long as we do not suppose it false.

Mr Christianson has no strong thesis of his own, unlike Arthur Koestler whose *Sleepwalkers* rightly aroused so much interest a few years ago (his ideas too are reflected in this book). Rather, Mr Christianson has attempted to synthesise recent writing in the history of science and particularly the interpretation of the Scientific Revolution. If he is not fully in control of his material this is in part because the history of astronomy has become—largely through the leadership exercised by Otto Neugebauer and his students—a highly technical subject, or rather one should say has been restored to its position as a highly technical subject.

In the personal aspects of his biographies Mr Christianson is on smoother ground, and he shows skill in bringing out the characters and intellectual qualities of his subjects in broad terms. If he sometimes stresses the melodramatic—Tycho's metal nose, Galileo's bastard daughters, Kepler's difficult mother and Newton's neuroses—this is what others have done before, including Koestler. For a philosopher like Galileo to have a mistress, and for a mathematician like Newton to have died a virgin is equally newsworthy. More seriously Mr Christianson presents a reasonably coherent and intelligible, if occasionally flawed portrait of his five heroes and conveys the integrity of the epoch that began with Copernicus and ended with Newton even if (as in any popular account) subtle nuances are absent.

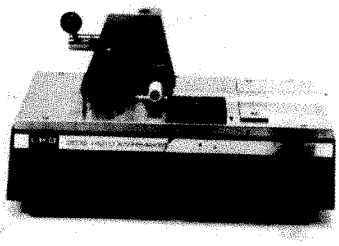
A. Rupert Hall

A. Rupert Hall is Professor of the History of Science and Technology at Imperial College, University of London, UK.

newly on the market

Knifemaker. LKB. The LKB histo knifemaker, the LKB 2078, quickly and routinely produces glass knives either 25 mm or 38 mm cutting-edge length using the 'Ralph knife' principle. Used in existing rotary or base-sledge microtomes, these reproducible, low-cost glass knives will produce large area, high quality, thin sections from either plastic- or paraffin-embedded specimens. The resulting improvement in resolution from the light microscope reveals wide new research and routine application areas in the histology laboratory.

Circle No. 31 on Reader Enquiry Card.



The LKB knifemaker

Environmental air monitor. Applied Science. The Bendix flasher has been introduced by Applied Science for environmental air monitoring by gas/chromatography. Volatile organics in environmental air are readily concentrated for GC analysis by pumping air through small packed adsorbent tubes. These organics can then be analysed by thermally desorbing them directly in a GC column with the Bendix flasher. Sub-p.p.b. range detectability of organics is possible. The flasher is ~0.5 cu ft and can be connected to any gas chromatograph. A portable air sampling pump, which attaches to the clothing of the user, is one of several accessories.

Circle No. 32 on Reader Enquiry Card.

Portable welding unit. British Rototherm. The 'little torch', miniature welding torch, introduced by British Rototherm can now be supplied as a complete kit comprising the torch, hoses, regulators, five tip sizes, goggles, spanners and trolley.

Circle No. 33 on Reader Enquiry Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Enquiry Card bound inside the cover.

X-ray energy spectrometer. Kevex. Nondestructive, non-contact, rapid multi-element analyses are performed with the Kevex 0750 X-ray energy spectrometer. With the 0750, solid, liquid or granular specimens may be rapidly analysed and compared with production control criteria. Analysis range: p.p.m. to 100% for most elements of atomic number 11–92 can be accomplished in minutes.

Circle No. 34 on Reader Enquiry Card.

Refrigerator incubator. Forma. A general purpose refrigerator incubator designed specifically for controlled temperature storage in the laboratory is now available from Forma Scientific. The unit is available with either a variable temperature range of 0–+60 °C, or factory set at +4 °C. Both models deliver dependable ±0.5 °C temperature control in a 17 cu. ft stainless steel cabinet insulated with 2″ of foamed-in-place urethane. Standard features include independent adjustable safety control with audible and visual alarm, door-ajar warning light with automatic blower cutoff, and automatic condensate removal.

Circle No. 35 on Reader Enquiry Card.

Ultramicrotome. LKB. The LKB Ultrotome V has greatly improved specifications and ergonomic lay-out of controls, a moveable microscope and click-stop (0.1 μm step) and macro (1 μm step) knife stage advance for serial thin and semi-thin sections. Section thickness and specimen approach are clearly and easily viewed using the single pre-positioned lighting system. The compact control unit is separate from the microtome unit thus allowing rapid, accurate changes to be made of all sectioning parameters during sectioning.

Circle No. 36 on Reader Enquiry Card.

Scalar irradiance meter. Biospherical. The QSL–100 is a sensitive new instrument which allows the accurate measurement of the total amount of light photosynthetically active in underwater and dry applications, from culture vessels to rain forests. Easily portable, the QSL–100 uses a solid Teflon sphere, 2 cm in diameter, optically connected to a hand-held detector, filter and amplifier assembly giving it an equal quantum response over 400–700 nm. The QSL–100 reads out the total number of photosynthetically active photons at the meter's tip when inserted into incubators or immersed in cell suspensions.

Circle No. 37 on Reader Enquiry Card.

Automatic conversion device for protein sequencers. Sequemat. The Sequemat P-6 Auto Converter is an in-line system for the conversion step of the Edman degradation. ATZ samples from each cycle collect in a reaction cell, convert to the PTH derivative and are delivered to the sequencer fraction collector. The P-6 unit has its own programmer for optimal flexibility of all functions. Benefits include improved yields of PTH derivatives and reduced operator error.

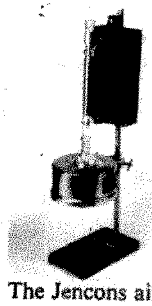
Circle No. 38 on Reader Enquiry Card.

Underwater acoustic television system. EMI. A portable acoustic television system has been developed by EMI Electronics which makes it possible to view objects underwater in conditions of zero visibility. With this 200-line scan system, the object to be investigated is irradiated with ultrasonic energy from an array of transducers arranged around the camera lens, and the reflected energy is focused on the face-plate of an image converter tube which converts the received signal to a visual display on a television monitor screen. It is also capable of locating cracks and voids on, or beneath, the surface of underwater structures. The underwater camera in its housing measures approximately 1,000 mm long by 250 mm in diameter, and weighs 20 kg. The system has a viewing range of up to 10 m and is capable of working at a depth of 300 m.

Circle No. 39 on Reader Enquiry Card.

Reflux condenser. Jencons. The airflux is a simple air-cooled reflux condenser which is as efficient as any normal water cooled condenser, but without the risks of flooding and its attendant fire risk, particularly when in use with solvents. The airflux needs no coolant water, and there are no parts to corrode or wear out. It has been operated for 1,500 h at laboratory temperatures up to 37 °C. Over this period there was no measurable loss of water from the boiling flask. Airflux can be used for distillation.

Circle No. 40 on Reader Enquiry Card.



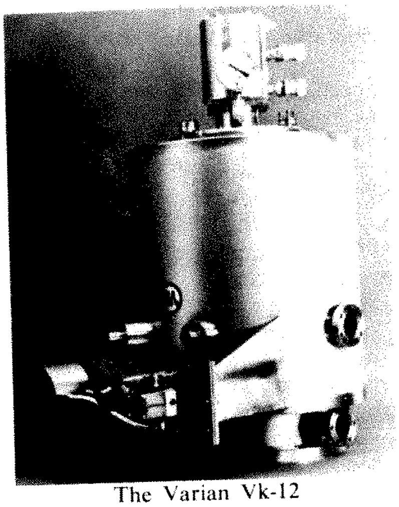
The Jencons airflux

Gas chromatograph. Packard. The Packard range of gas chromatographs now includes the 430 series GC with built-in dual integrator and printing facilities. Processor capacity has been expanded to perform independent dual-channel integration of the detector signals and to produce a complete analysis report via a built-in printer. When combined with the multi-dimensional switching system module, 18 complete GC programs of 15 time-based instructions each can be stored and executed at the touch of a button. The column switching technique employs solenoid valves which alter pressures at various points in the chromatographic system to change flow rates and reverse flow directions, thus eliminating leakage and overcoming the temperature limitations of conventional valves. The system has interchangeable analytical modules. Injection ports, columns and detectors are combined into one integral module, which can easily be changed to suit a particular analysis.
Circle No. 41 on Reader Enquiry Card.

Screening test for blood lead. MSE. An instrument is now available with which a blood sample can be checked in about 10 s to give an indication of the presence of lead in the body. The instrument, the ESA model 4000, uses fluorimetry to measure the amount of zinc protoporphyrin in a drop of blood (about 30 μl). Using the instrument, hundreds of blood samples can be checked in a day. The model 4000 is portable, and extremely easy to operate. The presence of lead in the environment during the analysis cannot affect the result.
Circle No. 42 on Reader Enquiry Card.

Liquid scintillation counter. Packard. A computer-controlled liquid scintillation counter where the only user interaction is loading and unloading trays of samples has been introduced by Packard. Automatic efficiency correlation ensures that all samples are measured with maximum Figure of Merit, plus optimum radionuclide separation in dual-label counting. The 15 different measuring programs are stored on a flexible diskette. Program selectors are clipped to the face of the 'servo-tray' by the user to ensure that correct parameters are retrieved from the diskette to measure the batch of samples. There are 9 trays, each with a capacity of 50 samples. Correlation curves are plotted, using a quench indicating parameter of external standard channels ratio, or a number derived from the measurement of an external standard source. An active parameter control allows modification to the parameters of a sample being measured to determine the best channel settings.
Circle No. 43 on Reader Enquiry Card.

Cryopump with integral valve mechanism. Varian. This is the first cryopump to incorporate a built-in valve mechanism designed specifically to meet the requirements of 'state-of-the-art' vacuum processes. Called the VK-12, the cryopump combines the performance of larger diffusion pump systems with the contamination-free operation of smaller ion pumps. It uses a unique integral swing gate valve mechanism that permits the seal plate to swing completely clear of the pumping orifice. A valve actuator controls the force required to achieve position sealing by swinging the seal plate into position and raising it against the sealing surface.
Circle No. 44 on Reader Enquiry Card.


The Varian Vk-12

Flatbed chart recorders. Bryans Southern. Bryans Southern Instruments have extended the range of flatbed chart recorders with their BS900 series of multi channel recorders. These are available in either three channel or four channel versions. Input amplifiers for each channel can be selected from modules having 12 or 18 voltage ranges or a 24 range module with 12 voltage and 12 current ranges; the chart speed module has 16 ranges. The recorder pens are rechargeable fibre tipped, write over the whole 250 mm width of the chart paper, and have a dynamic response of 0.65 s for full scale deflection.
Circle No. 45 on Reader Enquiry Card.

Laboratory furniture. Gallenkamp. A combination of traditional and modern timber-based materials, Gallenkamp System M furniture is robust, hard-wearing and attractive in appearance. The modular units are versatile and freely interchangeable. Most of the units are designed so that they can be packed flat. Modules available incorporate benches with a choice of drawer, cupboard or tray under storage units, sinks, mobile demonstration bench units and a fume cupboard.
Circle No. 46 on Reader Enquiry Card.

Dissolved hydrogen analyser. George Kent Electronic Products have introduced a dissolved hydrogen analyser, model 6421, to measure the amount of hydrogen in boiler steam. The new instrument gives advanced warning of boiler tube breakdown so that the boiler workload can be rescheduled to avoid failure. It works by measuring the amount of hydrogen dissolved in water condensed from the boiler steam. The instrument employs katharometric principles, and features a built-in power supply, automatic temperature compensation, dual ranges of 0–20 and 0–200 μg l^{-1}, a simple zero check which does not affect the sample flow and an adjustable level alarm signal output. It is unaffected by additives or contaminants.
Circle No. 47 on Reader Enquiry Card.

Monochromator. Jobin-Yvon. Jobin-Yvon have announced a new low-cost quarter-metre monochromator, the model H-25. This is a compact, lightweight and versatile instrument which can cover a very wide spectral range (from 180 nm in the near UV to 30 μm in the far IR) using high-efficiency holographic gratings. The gratings are easily interchangeable on pre-focused mountings. The optical arrangement is based on a Czerny-Turner mounting and uses two plane and two concave mirrors. The resolution is 0.3 nm at 500 nm while the dispersion is 3 nm mm^{-1}, with a 1,200 lines mm^{-1} grating.
Circle No. 48 on Reader Enquiry Card.

Protease detection kit. Bio-Rad. Bio-Rad's protease detection kit provides a convenient way to measure the activity of serum proteases, or of other proteases such as trypsin, α-chymotrypsin and pronase. When dissolved, tablets from the kit form an agar gel containing a protein preparation which gives it a turbid appearance. The sample to be assayed is placed in a well and diffuses into the gel forming a clear ring where the protein is digested. Proteolytic activity can then be determined very simply from the ring diameter, using guides provided in the kit.
Circle No. 49 on Reader Enquiry Card.

Electrophoresis power supplies. Buchler. Buchler Instruments has introduced two new DC power supplies designed for immuno-, paper and thin layer electrophoresis techniques. Each unit features easy to read, oversized meters and a single control for varying the output. One unit has ranges of 0–350 VDC, 0–100 mA and the other unit provides 0–1,000 VDC, 0–200 mA. Both of these units have dual output jacks so that more than one chamber may be operated at the same time.
Circle No. 50 on Reader Enquiry Card.

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THE OPEN UNIVERSITY
FACULTY OF SCIENCE
Course Co-ordinator in Physics

Applications are invited for the permanent post of Course Co-ordinator in the Physics Discipline.

The main duties of the post are to provide a range of academic, administrative and editorial services to course teams producing multi-media undergraduate courses in Physics. Applicants should have a first degree in Physics or a relevant subject, and an interest in administration.

Appointment will be made within the Course Co-ordinator salary scale £3,384 to £4,882 per annum with U.S.S benefits.

Application forms and further particulars are available from The Recruitment Office (JD1978/4), The Open University, P O Box 75, Walton Hall, Milton Keynes MK7 6AL, or by telephone from Milton Keynes 63404. there is a 24 hour answering service on 63868.

Closing date for applications January 3, 1979. 8706(A)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF THERAPEUTICS AND CLINICAL PHARMACOLOGY

Applications are invited from appropriately qualified scientists for a

SENIOR LECTURESHIP
in
THERAPEUTICS AND CLINICAL PHARMACOLOGY

The candidate will be expected to be an expert in pharmacokinetics and microanalytic techniques of measuring plasma drug levels and to work with medically qualified colleagues

The Department has beds in the Queen Elizabeth Hospital, Birmingham For a suitably qualified candidate the post may be associated with an honorary appointment as a Consulting Clinical Pharmacologist.

Salary in the range for non-clinical academic staff £7,506 to £9,263 per annum, plus superannuation.

Applications naming three referees, should be sent to the Assistant Registrar, Medical School, University of Birmingham, Birmingham B15 2TJ, by January 8, 1979

Further particulars may be obtained from Professor O. L Wade, Department of Therapeutics and Clinical Pharmacology, Clinical Research Block, Queen Elizabeth Hospital, Birmingham B15 2TH. 8738(A)

CHROMATIN RESEARCH
BIOPHYSICS LABORATORIES
PORTSMOUTH POLYTECHNIC
RESEARCH ASSOCIATE

required for two years initially, to work in Dr E. M. Bradbury's research group. The structure and function of chromatin are studied by both physical and biochemical methods The group has a high field N M.R spectrometer and is actively engaged in neutron and X-ray diffraction work. Experience of one of these techniques, although desirable, is not a prerequisite for appointment. However, knowledge of, and some research experience in general biochemical techniques is expected The successful candidate's project will depend on his/her interests and background.

Salary scale; £3,510 to £3,607 per annum.

Application forms and further details may be obtained from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth PO1 2QQ, to whom completed applications should be returned by December 30, 1978, quoting reference S54 8709(A)

DEPARTMENT OF HEALTH
NATIONAL HEALTH INSTITUTE
Wellington, New Zealand

Applications are invited from medical mycologists or science graduates with experience in medical mycology for a senior position in the Mycology Reference Laboratory.

The unit is responsible for the investigation of fungal infections, the identification of fungi referred from New Zealand and South West Pacific laboratories, for a fungal type culture collection and for the training of mycologists

The Mycology Reference Laboratory is used by medical laboratories throughout New Zealand as a source of reference and diagnostic service for fungal infections. It has working association with dermatologists, veterinarians, veterinary and university departments and overseas laboratories which specialise in mycology Qualifications desired are a medical or science degree with special training and experience in medical mycology Salary: Medical Graduate with science qualifications NZ$19,621 to NZ$25,364. Science Graduate NZ$10,672 to NZ$18,377 Each according to qualifications and experience. The post carries all privileges of State Services employment Applications should be forwarded with copies only of testimonials to The Chief Migration Officer, New Zealand House, Haymarket, London SW1Y 4TQ.

You should quote: IMM7/327/18

Assistance with fares and forwarding of baggage to New Zealand Closing date for applications: January 13, 1979 8690(A)

National Research Council Canada   Conseil national de recherches Canada

ASTRONOMER

The Herzberg Institute of Astrophysics has an opening for an **astronomer** at its **Dominion Astrophysical Observatory, Victoria, British Columbia.** The incumbent will be expected to undertake independent research in observational astronomy.

QUALIFICATIONS:

Candidates should have a Ph.D. or equivalent degree in astronomy or in a related discipline with several years of experience in astronomical research. Preference will be given to applicants with experience in extragalactic astronomy.

Knowledge of the English language is essential for this position.

Salary will depend on qualifications and experience.

Apply in writing giving complete details of education and experience to:

NRC

The Employment Officer, National Research Council of Canada, Ottawa, Ontario, Canada K1A OR6. Include a list of publications and the names of referees from whom confidential evaluations can be sought. In reply please quote HIA-56. 8702(A)

AGRICULTURAL RESEARCH COUNCIL
POULTRY RESEARCH CENTRE
KING'S BUILDINGS
WEST MAINS ROAD
EDINBURGH EH9 3JS

A BIOCHEMIST

with relevant physiological experience is required by the Reproductive Physiology Department at the above Centre, to undertake work in the field of reproductive physiology and biochemistry of the male Initially the appointee will be expected to participate in comparative investigation of the function of fowl and turkey spermatozoa associated with the development of techniques of semen storage above and below zero degrees centigrade A general interest in the function of the organs in the male concerned with reproduction and spermatozoa would also be desirable, relevant to maintaining the fertility of the male under different management systems In this respect facilities exist for collaborative work involving endocrinology and nutrition.

Qualifications—First or upper second class honours degree, at least two years' relevant post-graduate experience and, preferably, a Ph.D.

Salary—In scale £4,101 to £5,448 at H S C level and £5,154 to £6,898 at S.S.O level Entry at the higher level requires at least four years' post-graduate experience Non-contributory superannuation scheme. 22 days annual leave plus 10½ public/privilege holidays

Application forms can be obtained from the Secretary at the above address, these should be completed and returned to him not later than December 29, 1978. 8749(A)

THE UNIVERSITY OF BRITISH COLUMBIA

DEPARTMENT OF CHEMISTRY

ASSISTANT PROFESSORSHIP

Applications are invited from well qualified candidates for a position at the rank of **Assistant Professor** which will become vacant on June 30, 1979. Persons with research and teaching interests in one of the following areas will be given preference: **Organic Chemistry** including **Biological Organic Chemistry, Polymer Chemistry,** and **Analytical Chemistry.**

Applications consisting of a curriculum vitae, a list of publications and the names and addresses of three referees should be sent to the undersigned before March 31, 1979. This position is open to both male and female applicants.

Professor Charles A. McDowell
Department of Chemistry
UNIVERSITY OF BRITISH COLUMBIA
2075 Wesbrook Mall
Vancouver, British Columbia
Canada, V6T 1W5 8678(A)

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appointment to the following

RESEARCH SCHOOL OF PHYSICAL SCIENCES

DEPARTMENT OF NUCLEAR PHYSICS

FELLOW/SENIOR RESEARCH FELLOW/RESEARCH FELLOW/POSTDOCTORAL FELLOW

(3 posts)

The Department, with an academic staff of eighteen headed by Professor J O Newton, carries out research mainly in the field of nuclear structure physics but a small group also works in the area of ion-solid interactions Currently the nuclear physics interests are nuclear phenomena at very high angular momentum, measurement of static and dynamic electromagnetic moments, heavy-ion transfer reactions and nuclei far from the stability line The ion-solid interests are in beam foil spectroscopy, channelling and radiation damage The major accelerator is the 14UD pelletron tandem (14 MV terminal) An Enge split-pole spectrometer, equipped with focal plane detector, is available The Department hopes to be able to fill a tenured position (Fellow) and non-tenured positions (Postdoctoral Fellow, Research Fellow, Senior Research Fellow) in the area of nuclear physics; only applicants for non-tenured positions will be considered for the ion-solid field

CLOSING DATE **FEBRUARY 16, 1979.**

SALARIES Salary on appointment will be in accordance with qualifications and experience within the ranges Fellow $A17,694 to $A23,704, Research Fellow $A15,179 to $A19,813, Senior Research Fellow $A21,201 to $A25,289, Postdoctoral Fellow $A15,179 to $A19,813 per annum Current exchange rates $A equals $US1 13 £0 58

TENURE Appointment as Fellow is five years in first instance with the possibility, after review, of reappointment to retiring age 65 years, as Senior Research Fellow/Research Fellow normally for three years in the first instance with the possibility of extension to five years, as Postdoctoral Fellow for a maximum of two years

OTHER CONDITIONS Reasonable appointment expenses are paid Superannuation benefits are available for applicants who are eligible to contribute Assistance with housing is provided for an appointee from outside Canberra

The University reserves the right not to make an appointment or to make an appointment by invitation at any time

Prospective applicants should obtain further particulars from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF

8748(A)

UNIVERSITY OF SALFORD

DEPARTMENT OF BIOLOGY

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a Postdoctoral Research Fellow sponsored by the Science Research Council, to investigate the respiratory/cardiovascular function in terrestrial vertebrates under the supervision of Dr J H Brakenbury The appointment is tenable for three years commencing as soon as possible

Commencing salary within the range £3,883 to £4,328 per annum.

Application forms obtainable from the Registrar, University of Salford, Salford M5 4WT (Tel 061-736 5843, Ext. 215) to whom completed applications should be returned not later than Friday, December 22, 1978 quoting reference B/85/N. 8696(A)

UNIVERSITY OF IFE NIGERIA

Applicants are invited for the post of

SENIOR LECTURER

IN DEPARTMENT OF BIOCHEMISTRY

Salary scale N7,764 to N8,724 p a (£6,161 to £6,923 p a. sterling) (£1 sterling=N1 26) There may be supplementation of salary by £2,892 p a (sterling) for married appointees or £726 p a (sterling) for single appointees and provision of children's education allowances and holiday visit passages Fringe benefits include pension scheme or contract addition of 25% of salary (partly taxable), family passages, baggage allowances, car and housing allowances, various allowances

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent to Registrar, University of Ife, Ile-Ife, Nigeria by January 4, 1979

Applicants resident in the UK should also send one copy to the Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT Further details may be obtained from either address 8761(A)

AGRICULTURAL RESEARCH COUNCIL FOOD RESEARCH INSTITUTE

SCIENTIFIC SERVICES AND DEVELOPMENT DIVISION FRUIT AND VEGETABLE STORAGE GROUP

A SCIENTIST

is required as the senior member of the Fruit and Vegetable Storage Group which is part of the Scientific Services and Development Division

The person appointed will have joint responsibility for leading research, be responsible for the management of the experimental work and the overall supervision of the staff of the Group

The work will be concerned with monitoring the storage behaviour of soft fruits and vegetables and the performance of produce stores with the aim of developing improved techniques for chill storage

A first or upper second class Honours degree in some area of plant biology OR in food science and at least four years relevant postgraduate experience are required

Salary £5,154 to £6,898 Non-contributory superannuation scheme at age 18 years and over Five-day week and flexible working hours scheme operated

Application form and further particulars from the Secretary, Food Research Institute, Colney Lane, Norwich NR4 7UA, quoting Ref 78/2

Closing date, December 22, 1978

8762(A)

University of Sussex

SCHOOL OF BIOLOGICAL SCIENCES

TWO LECTURESHIPS IN BIOCHEMISTRY

Applications are invited for two posts of Lecturer in Biochemistry Candidates with research interests and teaching abilities in any field of Biochemistry will be considered, although preferred areas include bioenergetics, protein structure, biochemical endocrinology and regulation of cell metabolism The salary will be within the scale £3,883 to £7,754 per year (under review) with superannuation benefits

Application forms and further particulars can be obtained from the Assistant Secretary of Science, Science Office (E), Sussex House, University of Sussex, Falmer, Brighton BN1 9QG, to whom applications should be sent as soon as possible 8684(A)

ATHROFA GOGLEDD-DD CYMRU

THE NORTH E WALES INSTITUTE OF HIGHER EDUCATION

Clwyd Area Health Authority and the North E Wales Institute

RESEARCH TECHNICIAN—ANAESTHESIA AND IMMUNOCOMPETENCE

A Research Technician (Grade T2, Salary £3,369, Point 15—inclusive) is required for an initial two-year study of the effects of anaesthesia on lymphocyte function. This is a collaborative project between the North E Wales Institute and the Department of Anaesthetics, Maelor General Hospital, Wrexham

Applicants should be experienced in cell biology, immunology, biochemistry or haematology

Applications by letter (no forms) to the Institute Registrar, North E Wales Institute of Higher Education, Kelsterton College, Connah's Quay, Deeside, Clwyd Tel· Deeside 817531, by December 15, 1978 8755(A)

UNIVERSITY OF GLASGOW

INSTITUTE OF VIROLOGY

SENIOR LECTURESHIP IN VIROLOGY

Applications are invited for a Senior Lectureship in the Department of Virology at the Institute of Virology Tenable from March 1, 1979, or as soon as possible thereafter. The Institute, which is heavily engaged in biomedical research, comprises both the University Department of Virology and the Medical Research Council Virology Unit, under the direction of Professor J H Subak-Sharpe The successful applicant would have considerable opportunity for interaction and collaborative work with the interdisciplinary research staff In addition to teaching duties to undergraduates in B Sc and M B , Ch B courses, the applicant would have joint responsibility for the Diagnostic Virology Service to the Western Infirmary and Gartnavel Hospital Applicants should be medically qualified and have suitable specialist experience.

Salary will be according to placement on the University scale for clinical teachers The maximum of the Senior Lecturers' scale is £12,084

Further particulars may be obtaine from the Secretary of the Universit Court, (Room 18), University of Glasgow, Glasgow G12 8QQ, with whom applications (12 copies), giving th names and addresses of three referee should be lodged on or before Januar 10, 1979.

In reply please quote Ref. No 4317ML 8608(A)

THE UNIVERSITY OF NEWCASTLE

Newcastle, N S.W., Australia

POSTDOCTORAL RESEARCH FELLOW

DEPARTMENT OF MATHEMATICS

Field Renormalisation Group Theory in Statistical Mechanics

Project. The successful candidate will be familiar with the theory of Renormalisation Group methods and their application to Critical Phenomena, and will work on a project entitled "Investigation of non-translationally invariant lattice models by Renormalisation Group and Peturbation Expansion methods". In this project a study will be made of magnetic surfaces and films, polymer films and surfaces, percolation at a surface and in confined geometries, and related problems.

Qualifications A Ph D or the equivalent, preferably with some postdoctoral experience in the appropriate field.

Salary. Australian $A13,343 to $A16,882 per annum, plus travel allowance of up to $A2,000

Tenure. The appointment will be for one year in the first instance, with the prospect of renewal for two further years

Further scientific details of the project may be obtained from the Head of Department, Department of Mathematics, University of Newcastle, N S W., Australia, (Assoc Prof A J Guttmann)

Applications giving relevant personal and professional details, and the names of at least three referees should be sent to·

Vice-Principal,
University of Newcastle,
Newcastle, N S W 2308,
Australia 8674(A)

UNIVERSITY OF EAST ANGLIA

Norwich

HYDROLOGY AND FRESHWATER ECOLOGY

Posts are available for a Senior Research Associate (postdoctoral) and two Research Associates, (postgraduate) to assist in a major study of water chemistry, plankton ecology, and palaeolimnology of a part of the River Bure and its ten associated broads The senior post is for a hydrologist, preferably with experience of tracer studies, who will establish the patterns of water movement in the tidal river and broads and who will also be responsible for the day-to-day administration of the project The research associates will be graduates in environmental or biological sciences and will be concerned with chemical and biological aspects It is intended to produce a predictive model of the behaviour of the system which will aid in the eventual restoration of this very eutrophicated waterway Salary will be on scale 1A (senior post) £3,883 to £6,555 and scale 1B (research associates) £3,384 to £4,882 Applicants, who should be able to drive, should write to Dr Brian Moss, School of Environmental Sciences, University of East Anglia, Norwich NR4 7TJ, giving a curriculum vitae and quoting two immediately contactable referees, by December 15, 1978 8688(A)

RESEARCH FELLOW

A vacancy exists for the above post in the Department of Medical Cardiology, Royal Infirmary, Glasgow G4 0SF

The candidate will be mainly involved in developing and extending techniques to study various aspects of lipid and myocardial metabolism

The candidate will also be responsible for supervising the laboratory, which has excellent research facilities and has a staff comprising a research assistant and three technicians

Applications to Professor T D V Lawrie, at the Department of Medical Cardiology, from whom a detailed job description may be obtained

8701(A)

Rheumatism Research

Biologist/Biochemist

We require a post-doctoral scientist to lead a small established biological research group within the rheumatism research area in our laboratories in Nottingham. The group concentrates on investigating the biochemical and immunological aspects of chronic inflammatory disorders with the objective of developing new and more effective drugs

Applications are invited from graduates (men and women) who, ideally, have a first degree in Biochemistry or Cell Biology, a Ph.D in an appropriate subject and preferably with one or two years' relevant post-doctoral experience A knowledge of immunology would be an added advantage

This is an excellent opportunity for a person with initiative and the ability to motivate others to participate in an expanding area of our research

Company benefits include profit sharing and staff purchase privileges Generous help with relocation to the area is available.

Please send for application form to T. W Flower, Employment Manager (Technical),

The Boots Company Limited,

Station Street, Nottingham, NG2 3AA

8778(A)

UNIVERSITY OF NAIROBI

KENYA

Applications are invited for the post of PROFESSOR in the DEPARTMENT OF FORESTRY. Applicants should be holders of a basic degree in Forestry plus a Ph D in one of the professional disciplines of forestry in Silviculture, or Mensuration or Forest Engineering or Wood Technology The applicants should have several years' teaching experience at University level and several years' research in forestry science as supported by scientific publications Applicants with Masters degrees and several years' teaching and research experience at University may also be considered Appointee will be expected to teach undergraduate students in their particular field of interest in Forestry Science, to develop research, and to guide postgraduate students for higher degrees in Forestry Science Initial appointment will be on a two-year contract which can be renewed by mutual agreement.

Salary scale· K£4,632 to K£5,562 p a (K£1=£1 37 sterling) The British Government may provide salary supplementation of £5,796 p a (sterling) for married appointee or £4,182 p a (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages Family passages; S S S F or F S S U, non-contributory medical scheme; subsidised housing/housing allowance

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Registrar (Recruitment and Training), University of Nairobi, P O Box 30197, Nairobi, Kenya by January 3, 1979 Applicants resident in the U K should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT Further details may be obtained from either address

8722(A)

M.R.C. CLINICAL RESEARCH CENTRE

(Northwick Park Hospital)
WATFORD ROAD, HARROW
MIDDX. HA1 3UJ

Experienced virologist required in the Division of Perinatal Medicine to work in the sub division of embryology and foetal development The basic aim is to study the inter-relationship between oncogenic virus infection, host factor influence and consequential tumour development A knowledge and experience of immunology is desirable with an interest in immune responses to virus infection This is a limited term appointment for 4 to 5 years dependant upon age Salary within the range £5,367 to £7,754 plus London allowance

Application form and further details from Mrs J Tucker-Bull quoting ref. 133/1/3829/3957 Closing date December 30 8740(A)

PORTSMOUTH AND SOUTH EAST HAMPSHIRE HEALTH DISTRICT

SENIOR BIOCHEMIST

for the Renal/Endocrine Laboratory concerned with research and teaching of medical students Experience in Radioimmunoassay essential

Although based at St Mary's Hospital, Portsmouth, this is a joint appointment with Southampton University Applicants must possess a science degree and have at least 2 years experience as a basic grade Biochemist, or 5 years post graduate experience

Salary commencing at £5,451 per annum and rising annually to £6,837

For application form and job description ring 24 hour Ansaphone Portsmouth 22090 Completed applications should be returned to Personnel Officer, St Mary's Hospital, Portsmouth, Hants PO6 3AD

8726(A)

Department of Health and Social Security, London

Pharmacists/Biologists

...an important role in medicinal safety

The scientists appointed will join the medical and scientific secretariat serving the Committee on Safety of Medicines. They will assist senior medical staff in evaluating the safety and efficacy of new drug substances on which applications have been submitted for clinical trial certificates or product licences. Ample opportunity will be given to keep abreast of developments in this specialised area

Candidates, normally aged under 30, must have a good honours degree or equivalent in a biological science coupled with experience of pharmacology or toxicology. Preference will be given to candidates with a higher degree or to those who have had relevant experience in the pharmaceutical industry

Appointment as Higher Scientific Officer (£4560–£5910) or Scientific Officer (£3300–£4880) according to qualifications and experience. Promotion prospects Non-contributory pension scheme. *Please quote ref: SB/4/KB.*

■ *For application form see below*

Department of Health and Social Security, Middlesex

Pharmacist/Chemist

The British Pharmacopoeia Commission is responsible for all standards published in Britain for substances and articles used in human and veterinary medicine The pharmaceutical tests and methods of analysis included in their publications have largely been evolved in the Pharmacopoeia laboratory, which is equipped for a range of spectroscopy, chromatography, classical analysis and physical testing

As a member of a team of scientists, located in Canons Park, Middlesex, the pharmacist chemist appointed will critically examine proposed specifications for medical substances, prepare reports and make recommendations, maintain quality control measures for medicinal chemical reference substances used in assays and tests of the British Pharmacopoeia, and assist in the development of suitable analytical methods for routine quality control of pharmaceuticals In addition, the successful candidate will supervise and train junior staff in the team, and where necessary attend meetings to present reports and recommendations to the appropriate committees responsible for approving standards for publication

Candidates, normally aged under 30, should preferably have an honours degree or equivalent in chemistry or pharmacy. At least 2 years postgraduate experience of quality control of pharmaceuticals or the analytical techniques used in the laboratory is essential.

Appointment as Higher Scientific Officer (£4375–£5725) with starting salary according to qualifications and experience Promotion prospects. Non-contributory pension scheme. *Please quote ref. SB/12/KD*

For further details and an application form (to be returned by 5 January 1979) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours) *Please quote appropriate reference.*

ALL THESE POSTS ARE OPEN TO MEN AND WOMEN 8771(A)

Science Group CIVIL SERVICE

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(University of London)

Keppel Street, WC1E 7HT

DEPARTMENT OF HUMAN NUTRITION

LECTURER IN NUTRITION IN DEVELOPING COUNTRIES

Applications are invited for the above post, for which the main responsibilities will be —

(i) to provide advice, as required, on the nutritional aspects of projects and programmes in developing countries, and, in consultation with other members of the department and of the School, to formulate the appropriate nutritional inputs into such programmes,

(ii) to assist, both in the UK and overseas, in the training of nutritionists and nutrition field members,

(iii) to advance the subject by research.

Applicants should be medically qualified, should have experience of field work in developing countries, and should be freely available for overseas travel

The appointment will be initially for a period of 3 years, and will be in the salary range for clinical university lecturers (£4,986 to £7,393 and £354 London Weighting)

Application forms, which should be returned by January 31, 1979, and further information are available from F C Ball, Assistant Secretary 8763(A)

UNIVERSITY COLLEGE OF NORTH WALES

SCHOOL OF PLANT BIOLOGY

Applications are invited for the post of RESEARCH OFFICER, financed by the N.E.R.C to undertake research in collaboration with Dr C M Happey-Wood on the physiological ecology of micro-green algae Investigations will involve field work and laboratory studies of the effects of light and temperature on photosynthesis and growth, and of phosphorus uptake and flux in algal cultures under controlled conditions

This is a three-year project, but the appointment will be for one year in the first instance, on the lower portion of the Research and Analogous IA scale, £3,883 to £4,882 per annum

Applications (two copies) giving details of qualifications, age and experience, together with the names and addresses of two referees should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, from whom further particulars may be obtained

Closing date for the receipt of applications January 2, 1979

8758(A)

UNIVERSITY OF BATH

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a Postdoctoral Research Officer supported by the Science Research Council to work on the **structure and immunology of mammalian cell membranes** with particular reference to the glycoprotein components The milk fat globule membrane is being used as a convenient large scale source of mammalian membrane and its immunochemical characterisation is currently underway

The tenure of the post is one year Candidates should already possess a Ph D degree or expect to present their thesis shortly Experience in gas chromatography analysis of carbohydrates and/or immunochemistry would be an advantage The starting salary, which is not yet finalised, will be approximately £4,300

Application forms and further particulars are available from the Personnel Officer, University of Bath, Bath BA2 7AY, quoting reference number 78/261 N.

Informal contact may be made with Dr R Harrison, Tel: Bath 6941, Ext 674 or 522.

Closing date will be December 19, 1978 8675(A)

DUKE UNIVERSITY

DEPARTMENT OF CHEMISTRY
seeks appropriate candidates for the Reynolds Industries

PROFESSOR OF CHEMISTRY

an endowed professorship for a distinguished chemist committed to research in any area of chemistry.

Equal Opportunity/Affirmative Action Employer.

Inquiries should be sent to W. R Krigbaum, Chairman, P M. Gross Chemical Laboratory, Duke University, Durham, North Carolina 27706, by December 25, 1978.

8659(A)

DEPARTMENT OF NEUROLOGICAL SCIENCE

ROYAL FREE HOSPITAL

IMMUNOLOGIST

Re: Advertisement in *Nature*, November 30, (8628a) The corrected telephone number is 01-794 0500, extn 4363

8769(A)

UNIVERSITY OF NAIROBI KENYA

Applications are invited for the following posts in the DEPARTMENT OF CROP SCIENCE.—

PROFESSOR— HORTICULTURE OR AGRONOMY

Applications are invited from specialists in Agriculture, Horticulture or Botany who must be holders of a Ph D. either in Agronomy or Horticulture. Candidates should have considerable experience in teaching and research as substantiated by published work. The appointee will be required to undertake academic leadership in the field of Crop Science with regard to teaching and research both at undergraduate and postgraduate level The appointment will be on contract terms

PROFESSOR—CROP PROTECTION IN ENTOMOLOGY

Applications are invited from specialists in Agriculture, Horticulture or Zoology/Entomology who must be holders of a Ph.D. in Agricultural Entomology Candidates should have considerable experience in teaching and research as substantiated by publications in the relevant discipline The appointee will be required to undertake academic leadership in the field of Agricultural Entomology with regard to teaching and research at both undergraduate and postgraduate levels. In addition he/she will be required to lay groundwork in the design and implementation of a postgraduate course in Agricultural Pest Management This appointment will be on contract terms

Salary Scale. K£4,632 to K£5,562 p.a (K£1 equals £1.37 sterling) The British Government may provide salary supplementation of £5,796 p a (sterling) for married appointee or £4,182 p a. (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages Family passages, S S.S F or F.S.S U, non-contributory medical scheme; subsidised housing/housing allowance. Detailed applications (two copies) with curriculum vitae and naming three referees to be sent to Registrar (Recruitment and Training) University of Nairobi, PO Box 30197, Nairobi, Kenya by January 3, 1979. Applicants resident in the U K should also send one copy to Inter University Council, 90/91 Tottenham Court Road, London W1P 0DT Further details may be obtained from either address

8707(A)

Coleg Prifysgol Cymru

THE UNIVERSITY COLLEGE OF WALES

Aberystwyth

DEPARTMENT OF BIOCHEMISTRY AND AGRICULTURAL BIOCHEMISTRY

DEPARTMENTAL SUPERINTENDENT

(Technical Staff Grade 8)

Applicants should have considerable practical experience of the organisation and equipment needs of biochemical or chemical laboratories The person appointed will be responsible for the technical services of the Department and the relevant financial control.

Salary £4,746 to £5,160 per annum (under review).

Further details may be obtained from the Registrar, The University College of Wales, King Street, Aberystwyth, Dyfed SY23 2AX, to whom applicants should apply by letter by January 2, 1979

8677(A)

UNIVERSITY OF GLASGOW

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a Postdoctoral Research Assistant supported by the M.R C. to work with Dr H. G. Nimmo on the purification and regulatory properties of the mitochondrial glycerol phosphate acyltransferase from mammalian liver. Prior experience of working with membrane-bound enzymes would be an advantage

The appointment will initially be for one year, from January 1, 1979, renewable for two further years subject to satisfactory progress.

Salary within range £3,883 to £4,882 on Range 1A of the salary scale for Research and Analogous staff according to age and experience Superannuation Scheme will apply

Applications, with a curriculum vitae and the names of two referees, should be sent to Dr H. G. Nimmo, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, by December 22, 1978.

In reply please quote Ref No 4319M.

8641(A)

UNIVERSITY OF ZAMBIA

Applications are invited for the post of

SENIOR LECTURER/ LECTURER

in the
DEPARTMENT OF
LAND RESOURCES SURVEY—
SCHOOL OF NATURAL SCIENCES

Applicants should possess a first degree in Agriculture, Biology, Geography or Geology and preferably a higher degree in Land Resources Survey or an allied field. Appointee will be expected to teach the M Sc programme

Salary Scales Senior Lecturer, K7,608 to K8,106 p a., Lecturer, K4,956 to K7,608 p a (£1 sterling= K1 55.) The British Government may supplement salary in range £2,922 to £4,350 p a (sterling) for married appointee or £930 to £1,968 p a (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages Family passages, superannuation scheme, housing allowance, biennial overseas leave, various allowances.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent to Registrar, University of Zambia, P O Box 2379, Lusaka, Zambia, by January 13, 1979

Applicants resident in the U K should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT Further details may be obtained from either address.

8772(A)

Peptide Chemist

The growing importance of peptides in the search for
medicinal agents has placed a new emphasis on the
development of their large scale synthesis. As a
result of successful research in these laboratories
a position has been created for a
Peptide Development Chemist.
The successful applicant will preferably have several
years post-graduate experience in peptide research
or development. The laboratories are well equipped with all
the modern techniques associated with peptide
synthesis, purification and identification and the successful
applicant should be completely familiar in their use.
Terms and conditions of employment will be appropriate
to this appointment and salary will be negotiable
according to ability and experience plus assistance with
relocation to the Kingston-upon-Hull area
Applicants should write in complete confidence
to Miss N V McGeown, Personnel Officer,
at the address below:
Reckitt & Colman, Pharmaceutical Division,
Dansom Lane, Hull HU8 7DS

Reckitt & Colman

8667(A)

THE ROYAL MARSDEN HOSPITAL

Downs Road, Sutton, Surrey

TECHNICIAN

NUCLEAR MEDICINE DEPARTMENT

Salary
£2,700 to £3,621/£3,285 to £4,188

Technician required to complete a team of three in an in vitro laboratory carrying out mainly routine test involving radioisotopes The work is rewarding and requires an interest in patient contact.

Applicants should have 2 'A' Levels including a science subject and 3 years technical experience or ONC/HND

Application forms from Personnel Department, Royal Marsden Hospital, Fulham Road, London SW3 Telephone 01-352 8171, extn 446.

Closing date December 21, 1978

8764(A)

National Heart and Chest Hospitals

BROMPTON HOSPITAL

JUNIOR MEDICAL LABORATORY SCIENTIFIC OFFICER

required for Histopathology and Haematology Departments Candidates should have either 4 'O' levels (including Mathematics and 2 science subjects) or 2 'A' levels (including chemistry) or a degree

Application form available from Miss J. A Jenks, Personnel Manager, Brompton Hospital, Fulham Road, London SW3 6HP (tel: 01-352 8121, extn 4357).

8765(A)

GRADUATE or Postdoctoral Researc Assistant required for 1½ years in th Department of Microbiology for a M R C. research project on cyto megalovirus immunovirology. Labora tory experience in virology essential Applications, with full curriculum vitae and the names of two referees to the Secretary, Guy's Hospita Medical School, London Bridge SE 9RT quoting Ref. M C.2

8743(A)

UNIVERSITY OF DURHAM

DEPARTMENT OF CHEMISTRY

Applications are invited for a

POSTDOCTORAL SENIOR RESEARCH ASSISTANT

to work on a Ministry of Defenc project involving the evaluation of X ray photo electron spectroscop (E.S.C A.) for materials characterisa tion in collaboration with Dr D. T Clark The work will involve th application of E.S C.A. to a wide rang of problems in the surface characteris tion of materials of interest to th Materials Quality Assurance Directora of the Ministry of Defence. A back ground in either spectroscopy o materials science would be an advan tage. The appointment will be for on year from early in 1979.

Salary in the range £3,883 to £4,63 per annum, plus superannuation

Applications (three copies) should b sent by January 1, 1979 to th Registrar and Secretary, Scienc Laboratories, South Road, Durham DH1 3LE, from whom further particulars can be obtained.

8687(A)

M.R.C. CLINICAL RESEARCH CENTRE
(Northwick Park Hospital)

Watford Road, Harrow, Middlesex, HA1 3UJ

POSTDOCTORAL BIOCHEMIST

A Postdoctoral Enzymologist is required for investigations of the possible causes of perinatal deaths. (Many existing diagnostic categories are inadequate.) The programme would centre on developmental enzymology especially of mitochondrial systems and previous experience in biochemistry of mitochondria is therefore desirable. This appointment will be for three years only.

Salary within the range £4,408 to £6,080 and L.A.

Further details and application form from Mrs J Tucker-Bull, quoting reference 133/1/4267.

Closing date: December 30. 8766(A)

Reckitt & Colman

The recent growth in the research interests of our pharmaceutical division have given rise to a number of vacancies in our Biological Chemistry Department. The activities within this department are wide ranging and include bioanalysis and metabolism studies. These functions play a key role in the discovery and development phases of our drugs.

Biochemists: Drug Metabolism

The positions would suit graduate chemist biochemists preferably with several years relevant experience. The successful candidates will be predominantly involved with the use of radio labelled drugs for the investigation of their metabolic fate in animals and man.

Chemist/Biochemist: Bioanalysis

This position is suitable for a graduate with several years relevant experience or possibly a recently qualified PhD. He/she will assume responsibility for the development of radioimmunoassay techniques for new compounds. Experience of the synthetic problems associated with radioimmunoassay would prove an advantage. Commencing salaries will be negotiable and will reflect the qualifications and experience of the successful applicants. Application forms and further information regarding these posts may be obtained from Miss N.V. McGeown, Personnel Officer at the address below. All enquiries and applications will be treated in strict confidence.

Reckitt and Colman, Pharmaceutical Division,
Dansom Lane, Kingston upon Hull, HU8 7DS.

8693(A)

POSTDOCTORAL POSITION
Insect Biochemistry

Recent Ph.D. with strong background in protein fractionation, enzymology, and/or immunochemistry to study the role of juvenile hormone binding proteins and esterases in larval insect diapause. Twelve-month appointment available immediately (12,500). Send resume, names of three references to:

Dr G. M. Chippendale,
Entomology Department,
1-87 Agric. Bldg.,
University of Missouri,
Columbia, MO 65211, USA.

Equal Opportunity / Affirmative Action Employer. 8704(A)

BERKELEY PHYSICS

The Physics Department of the University of California, Berkeley, expects during the next two years to be making one or two appointments at the assistant professor level. Preferred areas of research are astrophysics, atomic and plasma physics, condensed matter physics, nuclear physics, and particle physics, with some priority given to theoretical particle physics.

Applicants should write before February 1, 1979, to the Chairman of the Physics Department, University of California, Berkeley, CA 94720, U.S.A. giving curriculum vitae, publication list, and references. The University of California is an Affirmative Action Employer. 8679(A)

UNIVERSITY OF NEWCASTLE UPON TYNE
DEPARTMENT OF AGRICULTURAL ENGINEERING
RESEARCH ASSOCIATE

Applications are invited for this post, funded by the Tropical Products Institute for a period of two years.

The work is connected with modelling heat and moisture movements in grain stored in unprotected metal silos subjected to diurnal heating, and experimental verification of the model. Overseas travel may be required.

Candidates should have a Ph.D. (or equivalent experience) in Physics, Chemical Engineering or a subject related to heat and mass transfer and with some computing experience. A knowledge of grain storage practice would be an advantage.

Starting salary within the range £3,883 to £4,382 per annum (under review). Duties to commence as soon as possible.

Applications together with curriculum vitae (three copies), quoting ref. Agric. 157 should be sent to the Registrar, University of Newcastle upon Tyne NE1 7RU, from whom further details may be obtained.

8691(A)

UNIVERSITY OF EXETER

Applications are invited for the post of Instrument Technician Grade 6 in the Department of Biological Sciences, to provide a comprehensive service of maintenance, repair and calibration of a wide range of scientific instruments. The work involves consultation with and advice to academic staff and postgraduates and instructing instrument users. A chemistry background would be an advantage.

Applicants should have an H.N.C. qualification and appropriate experience in the work. Further details can be obtained from the Laboratory Superintendent, Hatherly Laboratories, Prince of Wales Road, Exeter EX4 4PS.

Letters of application including the names and addresses of two referees should be sent to Miss Doreen B. Watson, University of Exeter by December 22, 1978. Please uote reference No. 5104. 8689(A)

TECHNICIAN/ RESEARCH OFFICER

required, from January 1979, to work on immunodiagnosis of leukaemia. Experience in fluorescence techniques, immunology or haematology an advantage.

Salary depending on qualifications within range £3,000 to £4,900.

For application form and job description please write or telephone Mrs Nelson, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2 on 01-242 0200, extn 305. 8768(A)

QUEEN ELIZABETH COLLEGE
Kensington
(University of London)
LECTURESHIP IN THE QUANTITATIVE ASPECTS OF MANAGEMENT

Applications are invited for the post of Lecturer in Management Studies to join a developing group within the Department of Food Science. Applicants will be expected to have an appropriate academic background with an original discipline in areas such as statistics, management science, mathematical economics, econometrics, operational research or economic geography. The successful applicant will be required to teach the application of quantitative methods to management problems to students following interdisciplinary degrees in the natural sciences and management studies. Outside contacts will be encouraged. Industrial or other relevant experience would be an advantage, and placing on the salary scale will reflect both qualifications and experience; the current scale (under review) is £2,909 to £7,754 per annum plus £450 per annum London allowance.

Application forms and further particulars are available from the College Secretary, Queen Elizabeth College, Campden Hill Road, London W8 7AH. Tel.: 01-937 5411, ext. 209. Closing date January 2, 1979. 8746(A)

UNIVERSITY OF WAIKATO
New Zealand
LECTURER OR SENIOR LECTURER IN EARTH SCIENCES

The University has a vacancy for a Lecturer or Senior Lecturer in the Department of Earth Sciences, available from February 1, 1979.

The University wishes to appoint a person with postgraduate qualifications involving at least one of the following: soil classification, soil survey, land use, soil genesis, soil chemistry, or soil physics. Experience in university teaching would be desirable but is not essential; however, the appointee would be expected to be interested in field research and to supervise postgraduate students in such research.

The current salary range for Lecturers is NZ$11,894 to NZ$14,488; and Senior Lecturers NZ$14,983 to NZ$17,145 to NZ$18,380 per annum.

Conditions of appointment and method of application are available from The Registrar, University of Waikato, Private Bag, Hamilton, New Zealand, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on December 20, 1978, but will be accepted for a further 14 days. 8747(A)

FULL PROFESSORSHIP OF BIOCHEMISTRY

available at the **University of Geneva Medical School, Geneva, Switzerland.**

Beside demonstrated abilities to lead an active research, the successful candidate should be able to teach in French.

Send complete resume to: Dr A. F. Muller, President of the Search Committee, Department of Internal Medicine, Hospital Cantonal, 1211 Geneva 4, Switzerland.

8724(A)

CENTRAL PUBLIC HEALTH LABORATORY

NATIONAL COLLECTION OF TYPE CULTURES

SYSTEMS DESIGNER/ PROGRAMMER

A science graduate is required for the computer operation of a bacterial identification service. Bacteria from clinical sources are identified by probalistic analysis of test results by reference to computer-stored data bases. Systems use Optical Mark Reader forms, Data Recognition (Dataterm 3) Machine punch tape terminal connected to the LUCS CDS time sharing system; current software in FORTRAN IV. There is considerable scope for improving the responsiveness of the system and the efficient storage and retrieval of data, and to revise the methodology or explore alternative methods.

Candidates with five years postgraduate experience may be appointed to the Senior Scientist Grade.

Salary on probationary scale £2,991 to £4,275 per annum, plus London Weighting, Post Probationary scale £3,798 to £4,899 per annum, plus London Weighting or Senior Scientific scale, if applicable, £5,451 to £6,837 per annum, plus London Weighting.

Applications to: Personnel Officer, Central Public Health Laboratory, Colidale Avenue, London, N.W.9. Tel: 01-205 7041 for a more detailed job description (Dr L. R. Hill on Ext: 47).

8676(A)

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF ANATOMY AND EMBRYOLOGY

RESEARCH ASSISTANT

(Salary Range IB)

quired for an M.R.C.-supported pro- ct involving electrophysiological udies of neuro-transmitter action on nooth muscle. The successful ap- icant will be supervised by Dr R. D. rves and encouraged to register for Higher Degree. Prior experience in urophysiology or an interest in ysics and electronics is desirable. ppointment for one year in the first stance, with the possibility of an tension to three.

Applications, including curriculum ae and names of two referees, to ofessor G. Burnstock, (N) Dept. of atomy and Embryology, University llege London, Gower Street, London CIE 6BT. 8695(A)

CSIRO

AUSTRALIA

Postdoctoral Research Fellow

Division of Soils

Narrabri, NSW/Canberra, A.C.T.

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: Pedology

General: The Division with headquarters in Adelaide has, through its Canberra Laboratories, a major interest in soil research in the fluviatile landscapes of the main river systems of NSW both in their middle and upper reaches (the water sources areas) and in the alluvial plains where irrigation and intensive agriculture are practised. The predominantly clay soils of the alluvial plains have varying cropping reputations. Some, as in the Namoi Valley in which cotton is grown, are thought to pose management problems associated with structural instability which leads to water logging and aeration deficiencies. A prerequisite for tackling such problems is a though understanding of the nature and properties of the soils and their distribution in the landscape. This provides a basis for examining changes wrought by current practices and for proposing the appropriate management modifications. It can also ensure that field trials are located on representative sites, and enables the confident extension of results of such trials.

Duties: To study the soils, their properties and distribution in relation to landscape in the Lower Namoi Valley and to report on the nature of the interaction between soil features and current systems of management. Attention will be directed to placing these soils in the context of other irrigated clay soils of Eastern Australia.

Qualifications: A Ph.D degree in an appropriate field or postgraduate research experience of equivalent standard supported by satisfactory evidence of research ability.

Note: The Division of Plant Industry in collaboration with the NSW Department of Agriculture has a field research station at Myall Vale, near Narrabri and will provide back-up facilities for the field studies.

Location: Initially the appointee will be stationed at Narrabri where a house is available for rental. Subsequently the appointee will be located in Canberra.

Salary: Research Scientists or Senior Research Scientists: A$14,829—A$21,543 p.a.

Tenure: A fixed term appointment of 5 years.

Applications **IN DUPLICATE,** stating **FULL** personal and professional details, the names and addresses of at least two professional referees, and **QUOTING REFERENCE NUMBER 270/480** should reach:—

The Personnel Officer,
Australian Scientific Liaison Office,
Canberra House,
Maltravers Street,
LONDON,
WC2R 3EH.

by 6th January, 1979.

Applications in U.S.A. and Canada should be sent to:—

The Counsellor (Scientific),
Embassy of Australia,
1601 Massachusetts Avenue, N.W.,
WASHINGTON, D.C., 20036,
U.S.A.

8742(A)

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(Universtiy of London)

Keppel Street WC1E 7HT

PREVENTIVE TERATOLOGY UNIT

Applications are invited for a

MEDICAL LABORATORY SCIENTIFIC OFFICER IN BIOCHEMICAL TERATOLOGY

to work on congenital malformations associated with environmental agents.

The appointment is for up to 18 months at a salary, depending on age, experience and qualifications, on a scale which rises to £3,930 plus £354 London Weighting.

Applications, stating qualifications and experience and the names of two referees, should be sent to the Secretary (A1) at the School. 8745(A)

M.R.C. CLINICAL RESEARCH CENTRE

(Northwick Park Hospital)

WATFORD ROAD, HARROW

MIDDX. HA1 3UJ

The division of immunological medicine (Head: Dr G. L. Asherson) has a vacancy for imaginative SCIENTIST with biochemical or biological interests to study T suppressor cells and their assay in animals and man.

Salary within the range £3,909 to £7,754 plus L.A. This will be a short term appointment for 3 years.

Candidates may visit the department by telephoning G. L. Asherson 01-864 5311 extn 2691 or further details and application form obtainable from Mrs J. Tucker-Bull, quoting reference 118/1/4194.

Closing date: January 3, 1979.

8767(A)

EUROPEAN MOLECULAR BIOLOGY LABORATORY

IMAGE PROCESSING

Applications are invited for a post in the Instrumentation Division to develop and maintain a user-oriented suite of image-processing programmes, particularly for the analysis of electron micrographs of biological specimens. Extensive experience in the writing, documentation and maintenance of programmes for large image processing problems is necessary. A background in the development of efficient mathematical algorithms for image analysis and a familiarity with applications of electron microscopy in biology is desirable. Experience with small and medium computer configurations, their operating systems and I/O software/hardware writer/user will be useful.

The successful applicant will implement the programmes on a NORD 10/NORD 50, interfaced with an HB5 STEM and driving an FPS array processor and extensive interactive display and I/O systems. There will be remote access to a DEC 1090. Research activities will include the development of new mathematical algorithms to analyse two- and three-dimensional data.

The salary offered will be between DM 3400 and DM 4300 depending on age, qualifications and experience.

Applications, with a curriculum vitae and the names of referees, and quoting reference 78/40, should be sent to:

Sir John Kendrew
Director General
Postfach 10.2209
6900 Heidelberg
Germany

8710(A)

UNIVERSITY COLLEGE DUBLIN

Applications are invited by the Governing Body of the College for the following full-time statutory post:

PROFESSORSHIP OF ORGANIC CHEMISTRY

Prior to application, further information (including details of application procedure) should be obtained from the Secretary and Bursar, University College, Belfield, Dublin 4. Telephone enquiries: 693244, Ext. 431.

The latest date for receipt of completed applications is **Thursday, February 8, 1979.** 8666(A)

We are a major pharmaceutical company with our own large research division.

We require a qualified

neuropharmacologist

as head of our new neuropharmacological laboratory.

Applicants should send a detailed curriculum vitae to

Peter Assent
c/o Grünenthal GmbH
Postbox 1 29
5190 Stolberg Rhld.
Germany

8723(A)

UNIVERSITY OF EDINBURGH

DEPARTMENT OF MOLECULAR BIOLOGY

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the post of Postdoctoral Research Fellow to work on a project funded by the S.R.C. The project is concerned with the separate isolation of copy DNA sequences from *Xenopus* oocyte messenger RNA by molecular cloning and applicants should have experience of nucleic acid research and/or molecular cloning techniques.

The salary is on Range IA (£3,883 to £6,555 per annum) and the post is for three years.

Applications, including a curriculum vitae and the names and addresses of two referees, should be sent to Dr P. J. Ford, Department of Molecular Biology, King's Buildings, Mayfield Road, Edinburgh EH9 3JR. Please quote Reference 5035. 8610(A)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

DEPARTMENT OF BIOLOGY

FISHERIES BIOLOGY

Applications are invited for a position open at senior rank, in the area of Fisheries Biology. Candidates must demonstrate a strong record of research and publication in fisheries, and a successful background in supervision of fisheries research at the M.Sc. and Ph.D. levels. The successful candidate will play a key role in development of a new fisheries program.

ASSISTANT PROFESSOR—AQUATIC ZOOLOGY

Applications are invited for a position open at the rank of Assistant Professor, in the area of Aquatic Zoology. Enquiries are especially welcome from candidates with specialisation in any of the following areas: marine and freshwater fishes; limnology; planktology; fish population dynamics.

Applicants should submit a *curriculum vitae* together with the names of three referees. Applications close on **January 31, 1979** with Dr G. R. South, Head, Department of Biology, Memorial University of Newfoundland, St John's, Newfoundland A1B 3X9, Canada. 8719(A)

UNIVERSITY OF NAIROBI KENYA

Applications are invited for the post of PROFESSOR in the DEPARTMENT OF ANIMAL PRODUCTION. Candidates should hold a Ph.D. and have considerable experience in teaching at University level, in either Animal Breeding, Animal Management or Animal Nutrition and Feeding. The appointee will be expected to teach both undergraduate and postgraduate students and advise the department on the syllabus currently in force. The minimum period for the appointment is one year.

Salary Scale: Professor K£4,632 to K£5,562 p.a. (K£1=£1.37 sterling). The British Government may provide salary supplementation of £5,796 p.a. (sterling) for married appointee or £4,182 p.a. (sterling) for single appointee (reviewed annually and normally free of all tax) and provide childrens education allowances and holiday visit passages. Family passages: S.S.S.F. or F.S.S.U.; non-contributory medical scheme; subsidised housing/housing allowance.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Registrar (Recruitment and Training), University of Nairobi, P.O. Box 30197, Nairobi, Kenya by January 3, 1979. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

8720(A)

THAI NATIONALS

Faculty positions available for graduates in biochemistry or related fields. Good opportunity to join a young dynamic department with active programs in research (currently: nutrition, reproduction and tropical diseases) and teaching (medical and postgraduate students). Special consideration to those trained in biotechnology, cancer and endocrinology. Send curriculum vitae to **Chairman, Department of Biochemistry, Faculty of Science, Mahidol University, Rama VI Rd., Bangkok, Thailand.**

8717(A)

THE ROYAL HORTICULTURAL SOCIETY

Appointment of Botanist

Applications are invited for the post of Botanist at the Society's Garden at Wisley.

The duties will include advisory work on plant identification, and physiology, genetics and chemistry related to horticulture; demonstrations to trainees, cataloguing and labelling plants at Wisley and Committee work. Graduates in botany or horticulture are preferred.

The salary, reviewed annually, will be related to qualifications and experience. The post is pensionable.

Applications with details of qualifications and experience and the names of two persons to whom reference may be made should be sent so as to reach the Secretary, Royal Horticultural Society, Vincent Square, London SW1P 2PE, by Monday January 15, 1979.

8716(A)

BONN UNIVERSITY and MAX-PLANCK-INSTITUTE FOR RADIOASTRONOMY

Three postdoctoral research positions are open at the

SONDERFORSCHUNGSBEREICH RADIOASTRONOMIE

in theoretical astrophysics (interstellar medium, galactic evolution, pulsars) for one year, possibly three years. SALARY: 40,000 to 48,000 DM per annum. Applications including curriculum vitae, bibliography and the names of two referees should be sent to:

Dr W. Priester, Sonderforschungsbereich Radioastronomie, Auf dem Huegel 71, D 5300 BONN 1, Federal Republic of Germany. 8715(A)

PLANT BREEDING INSTITUTE

Maris Lane, Trumpington
Cambridge CB2 2LQ

HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER

CYTOGENETICS DEPARTMENT

A Molecular Biologist is require to participate in and be responsibl for parts of a programme of studyin plant chromosomes, isolating plan genes and producing vectors an specific nucleotide sequences for th transformation of plant cells.

Candidates should have a 1st o Upper 2nd Class Honours Degree i an appropriate biological science an at least two years' postgraduate experience in molecular biology o related fields of biochemistry.

The appointment will be as Highe Scientific Officer (£4,101 to £5,448 pe annum) or Senior Scientific Office (£5,154 to £6,898 per annum). At leas four years' postgraduate experience i required for appointment as Senio Scientific Officer.

Applications with curriculum vita and the names and addresses of thre referees should be sent to the Assista Secretary (Establishment) by Decembe 22, 1978.

Please quote reference CYT/35.

8712(A)

UNIVERSITY OF IFE NIGERIA

Applications are invited for the following posts in the Faculty of Pharmacy:—

1. PROFESSOR IN DEPARTMENT OF PHARMACOGNOSY (incorporating DRUG RESEARCH UNIT).
2. PROFESSOR IN DEPARTMENT OF PHARMACEUTICALS. Candidates should have considerable teaching and research experience in the field of Pharmaceutical Technology or General Pharmaceutics.

Salary Scale: Professor, N11,268 to N12,420 p.a. (£8,942 to £9,857 p.a. sterling). (£1 sterling=N1.26.) There may be supplementation by £2,160 p.a. (sterling) for married appointees or £Nil p.a. (sterling) for single appointees and provision of children's education allowances and holiday visit passages. Fringe benefits include pension scheme or contract addition of 25% of salary (partly taxable); family passages; baggage allowances; car and housing allowances; various allowances.

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent to Registrar, University of Ife, Ile-Ife, Nigeria, by January 4, 1979.

Applicants resident in the U.K. should also send one copy to the Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 8774(A)

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for a post of DEMONSTRATOR from candidates who have, or have submitted for, a Ph.D. in Biochemistry, for a period up to three years, tenable as soon as possible. The duties will involve participation in departmental teaching and in one of the research projects currently in progress. These include membrane transport of nucleic acid precusors, the role of folding units in enzyme structure, DNA repair and genetic recombination in E. coli, genetic engineering, triglyceride and lipoprotein metabolism, enzymology of resistance in antibiotics, lactose transport and bioenergetics in E. coli.

Salary will be at an appropriate point on the Grade 1B (Bar) scale: £3,384 to £4,882 per annum, according to age, qualifications and experience. Membership of the appropriate University superannuation scheme will be required.

Further particulars may be obtained from the Registrar, The University, Newcastle upon Tyne NE1 7RU with whom applications (two copies), together with the names and addresses of three referees should be lodged not later than December 21, 1978. Please quote reference N. 8685(A)

THE ROYAL SOCIETY

The Royal Society requires a science graduate (up to 25-years-old) to assist in its editorial department. General aptitude for scientific editorial work required; sound grounding in mathematics and knowledge of biological nomenclature necessary. Progressive salary scale and superannuation scheme. Commencing salary up to £4,395 per annum depending on age and experience. Applications with names of two referees to the Executive Secretary, 6 Carlton House Terrace, London SW1Y 5AG by January 2, 1979, marking the envelope 'Personal W.G.E.'. 8729(A)

TECHNICIAN (Grade 5) required in Department of Microbiology, University of Reading. Main duties include supervision of laboratory services in section dealing with teaching and research in Microbial Physiology and Biochemistry; equipment for teaching and research; chemical stores. Salary according to qualifications and experience in range £3,186 to £3,720 p.a. (under review). Further details available on request. Apply, stating qualifications and experience and giving the names of two referees, quoting Ref. T.N. 74A, to Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading RG6 2AH. 8751(A)

HOUGHTON POULTRY RESEARCH STATION

GENETICIST

A vacancy exists in the Leukosis Experimental Unit for a geneticist to study the inheritance and nature of resistance of chickens to oncogenic and other viruses, and to assist in the formulation of breeding programmes for the Institute's flocks.

Applicants must have a first or upper second class honours degree, with genetics as a major subject in the first or a higher degree, and preferably a Ph.D. or at least 3 years' relevant postgraduate experience. A minimum of four years' relevant postgraduate experience is required for the S.S.O. grade.

The appointment will be to the Higher Scientific Officer grade (£4,101 by 8 to £5,448) or the Senior Scientific Officer grade (£5,154 by 8 to £6,898), grade and starting salary depending upon experience. Non-contributory superannuation scheme.

Further particulars and application forms from the Station Secretary, Houghton Poultry Research Station, Houghton, Huntingdon, Cambs. PE17 2DA. Closing date for applications is January 10, 1979.

8671(A)

MASSEY UNIVERSITY

Palmerston North, New Zealand

LECTURESHIP IN BOTANY

The Department of Botany and Zoology has a vacancy for a botanist with a general interest in the teaching of biology and research interests in developmental plant physiology and/or morphology. However, all applicants with interests in experimental plant science will be considered.

The successful candidate who will preferably have a Ph.D. may be expected to contribute to courses in plant morphology, morphogenesis, developmental plant physiology, developmental biology and cytology. The Botany and Zoology Department is well-equipped for experimental and field research. The Department is involved in teaching at all levels within the University, and has increasing commitments to extra-mural courses serving the whole of New Zealand.

Salary NZ$11,894 to $14,488.

Further details of this position, and of this University, and Conditions of Appointment, may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or the Registrar of the University.

Applications close on **January 19, 1979.** 8718(A)

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOCHEMISTRY

RESEARCH TECHNICIAN

(Grade 3)

required for Monoclonal Antibody Production and Biochemical Investigations of Erythrocyte Proteins, particularly those affected by Heredity Spherocytosis, to work in a new well-equipped reserch group investigating cell membrane diseases. A sound biochemical background and previous experience in tissue culture, protein chemistry, electrophoresis and/or chromatography are disirable.

The appointment is from January 1, 1979 initially until July 31, but extendable subject to continuing support from granting agencies, at a starting salary of £3,153 (under review) including London Weighting.

Write or telephone soon for an application form to the Departmental Superintendent, Biochemistry Department, Imperial College, London SW7 2AZ. Tel: 01-589 5111 Ext. 1119, quoting reference DMS RT3.

8713(A)

WILTSHIRE Area Health Authority

Salisbury Health District

Scientific Officer

(Basic Grade)

required to work in the Burns Research Unit at Odstock Hospital, Near Salisbury. This post is suitable for a graduate in Science (or equivalent qualifications) with an interest in studying the structure, metabolism and response to injury of human tissues and organ systems. The appointee will undertake laboratory work under the supervision of the Principal Scientific Officer (Dr P. G. Shakespeare), but there will be opportunity to develop their own ideas within the sphere of interest of the laboratory.

Application Forms and Job Descriptions available from District Personnel Department, Odstock Hospital, Salisbury, Wilts. Closing date 31st December, 1979. 8694 (A)

Western Australian
Institute of Technology

RESEARCH ASSOCIATE – ISOTOPE GEOCHRONOLOGY

DEPARTMENT OF PHYSICS

Applicants will be required to work on the establishment of the Sm-Nd geochronological method and its application to a range of problems in the development of the Archaean crust of W.A. The department has continuing Rb-Sr program in association with the Geological Surveys of W.A., and proposes to supplement this with Sm-Nd work as an integral part of this joint geochronological research.

Applicants require a recently completed higher degree in isotope geochronology or closely aligned discipline, and must have shown independent analytical ability.

Applicants required to commence work in Perth early in 1979.

APPOINTMENT: at an annual salary $13,200, with limited relocation expenses will be for two years. Appointment at a higher level may be possible for applicants with substantial direct experience of Sm-Nd techniques.

APPLICATIONS: with full personal details and the names and addresses of two referees, should be submitted by 22nd December, 1978 to K. Rosman, Department of Physics, Western Australian Institute of Technology, Hayman Road, South Bentley, 6102, Western Australia.

8673(A)

AN FORAS TALUNTAIS

requires

AGRICULTURAL SCIENCE GRADUATES (2)

to participate in the dairy husbandry research programme of the Moorepark Research Centre, Fermoy, Co. Cork. One appointment will be up to and including Principal Research Officer level (salary minimum £9,387), the other up to and including Senior Research Officer level (salary maximum £8,465). Marriage and Children's allowances, Non-contributory Pension Scheme and provision for Widows' and Orphans' pensions.

ESSENTIAL: An Honours University Degree or equivalent in Agricultural Science.

DESIRABLE: Suitable research or other relevant experience.

Application forms and further particulars from Personnel Department (ref. M.O.R.), An Foras Taluntais Headquarters, 19 Sandymount Avenue, Dublin 4 (Phone: 01-688188). Latest date for receipt of completed forms: January 5, 1979. 8699(A)

There is a vacancy for a BIOLOGIST in *Nature's* London office. He or she would join the team which assesses the suitability of biological manuscripts for publication; there would also be opportunities to work with members of the *Nature* staff in compiling other sections of the journal. The job might appeal to the holder either of a bachelor's or an advanced degree.

Write to:
The Editor
NATURE
MACMILLAN JOURNALS LIMITED
4 Little Essex Street
London WC2R 3LF

enclosing a curriculum vitae and any other material you consider relevant.

8577(A)

ST JAMES'S HOSPITAL, LEEDS, YORKS

Applications are invited from candidates with an Honours degree in one of the Biological Sciences for the post of SCIENTIFIC OFFICER in the Regional Cytogenetic Unit established at this hospital. The Unit undertakes the cytogenetic investigations required for patients within the area covered by the Leeds Regional Health Authority.

Salary: Starting at £3,486 rising annually to £4,275.

Applications, giving the names and addresses of two referees should be submitted to:

Central Personnel Office
St James's University Hospital
Beckett Street
Leeds LS9 7TF

Closing date for receipt of applications: December 28, 1978. 8750(A)

THE DIVISION OF
ENTERIC PATHOGENS
has a vacancy for a

MEDICAL LABORATORY SCIENTIFIC OFFICER

Suitable qualifications would be a relevant Science degree, A.I.L.M.S. or H.N.C. with experience in Microbiology.

The successful applicant will work in the Drug Resistance Research Unit, which is concerned with studies on the epidemiology and ecology of transferable drug resistance in the enterobacteria.

Applications to: Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT. Tel: 01-205 7041. 8744(A)

UNIVERSITY OF OXFORD

University Lectureship in Atmospheric Physics

Applications are invited from persons with a background in atmospheric physics, dynamical meteorology or geophysical fluid dynamics for the above post in the Department of Atmospheric Physics. The successful candidate may be offered a tutorial fellowship by Merton College. Details of the University and College appointments may be obtained from Professor J. T. Houghton, F.R.S., Department of Atmospheric Physics, Clarendon Laboratory, Oxford University, to whom completed applications should be sent by January 31, 1979.

8732(A)

UNIVERSITY OF NAIROBI KENYA

Applications are invited for the following posts in the

DEPARTMENT OF AGRICULTURAL ENGINEERING

PROFESSOR

Applicants should have a Ph.D., or at least an M.Sc., in agricultural engineering plus several years' teaching experience at University level and knowledge of development of farm power and machinery systems for tropical agriculture in developing countries. Research experienre in farm power and machinery would also be an advantage. The appointee is expected to take a leading role in the development of teaching and research in the required specialism. The teaching will be at both undergraduate and postgraduate levels to both agricultural and agricultural engineering students. The appointment will be on contract terms.

PROFESSOR (SOIL AND WATER ENGINEERING)

Applicants should have a Ph.D., or at least an M.Sc., in agricultural engineering, hydrology or a related field. Several years' teaching experience at university level in the development methods for soil and water conservation in developing countries is essential. Research experience in soil conservation engineering or soil and water management, would be an advantage. The appointee is expected to take a leading role in the development of teaching and research in the indicated fields. The teaching will be at undergraduate and postgraduate level to both agricultural and agricultural engineering students. The appointment will be on contract terms.

Salary scale: K£4,632 to K£5,562 per annum (K£1 equals £1.37 sterling). The British Government may provide salary supplementation of £5,796 per annum (sterling) for married appointee or £4,182 per annum (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages; S.S.S.F. or F.S.S.U.; non-contributory medical scheme; subsidised housing/housing allowance. Detailed applications (two copies) with curriculum vitae and naming three referees to be sent to Registrar (Recruitment and Training), University of Nairobi, P.O. Box 30197, Nairobi, Kenya by January 3, 1979. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 8708(A)

PLANT BREEDING INSTITUTE

Maris Lane, Trumpington
Cambridge CB2 2LQ

HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER

CYTOGENETICS DEPARTMENT

A Cell Biologist is required for a period of three years to carry out a feasibility study of transferring DNA into cereals and related species using techniques involving cell or tissues other than cell protoplasts where whole plant regeneration may be a problem.

Candidates should have a 1st or Upper 2nd Class Honours Degree in an appropriate biological science and at least two years' postgraduate experience in cell biology or related research including practical experience of micromanipulation techniques.

The appointment will be as Higher Scientific Officer (£4,101 to £5,448 per annum) or Senior Scientific Officer (£5,154 to £6,898 per annum). At least four years' relevant postgraduate experience is required for appointment as Senior Scientific Officer.

Applications with curriculum vitae and the names and addresses of three referees should be sent to the Assistant Secretary (Establishment) by December 22, 1978.

Please quote reference CYT/34.

8711(A)
8733(A)

Avon Area Health Authority (T)

Southmead Health District

SOUTHMEAD HOSPITAL

Bristol

SENIOR BIOCHEMIST

Applications are invited from suitably experienced persons for the above post in the Department of Clinical Chemistry. The Department has a Consultant Chemical Pathologist and a Top Grade Biochemist and serves a large District General Hospital which also carries out clinical teaching for the University of Bristol. The laboratory is well equipped and has a variety of research interests, mainly in the fields of paediatric biochemistry and immunoassays. The person appointed will probably be expected eventually to take day-to-day responsibility for the steroid/infertility section of the laboratory under the direction of the Principal Biochemist. This section performs a range of steroid analyses and provides an Area service for foeto-placental function tests and infertility investigations.

Further information available from: Dr D. J. Goldie (Tel: 0272 505050, Ext: 51) or Dr J. B. Holton (Tel: 0272 505050, Ext. 52).

Application forms obtainable from: The District Personnel Officer, Southmead Hospital, Bristol BS10 5NB. (Tel: 0272 505050, Ext. 162) and should be returned by December 31, 1978.

8725(A)

POSITION AVAILABLE IMMUNOLOGIST/IMMUNOPATHOLOGIST

The Department of Pathology, Hematopathology Section, University of Southern California School of Medicine, is seeking applicants who have recently completed a Ph.D. program in the areas of immunology/immunopathology for full-time appointment as research associate/scholar. Applicants should be qualified in the areas of cellular immunology and tissue culture and should have experience with mice. Salary negotiable. Following appointment qualified individuals will be encouraged to seek faculty status. Please send curriculum vitae, description of present and future research and names of references to: Dr Paul K Pattengale, U.S.C. School of Medicine, 2025 Zonal Avenue, Los Angeles, CA 90033.

Affirmative Action/Equal Opportunity Employer. 8703(A)

GLASSHOUSE CROPS RESEARCH INSTITUTE

requires SCIENTIFIC OFFICER/HIGHER SCIENTIFIC OFFICER in **PLANT BREEDING DEPARTMENT** for work on genetic control of lateral shoot initiation and development in tomato. Applicants should have First or Upper Second class Honours degree in a biological subject, with specialisation in genetics supported by biochemistry and plant physiology. Salary within scale £2,839 to £4,415 (S.O.) or £4,101 to £5,448 (H.S.O.) according to qualifications and experience. At least two years relevant postgraduate experience required for appointment as H.S.O. Non-contributory superannuation scheme. Further details available. Applications giving full biographical details and names and addresses of two referees to Secretary, Glasshouse Crops Research Institute, Worthing Road, Rustington, Littlehampton, West Sussex BN16 3PU by January 2, 1979.

8741(A)

BEATSON INSTITUTE FOR CANCER RESEARCH

SCIENTIST

A vacancy exists for a scientist to join a team studying the role of RNA tumour viruses in differentiation and transformation. The appointment is on a new five-year M.R.C. programme. Applicants should have experience in the general area of Cell Biology/Molecular Biology. Salary will be on the University of Glasgow scales for Research Staff and will be negotiable according to experience. For further information contact Prof. W. Ostertag or Dr I. B. Pragnell, Beatson Institute for Cancer Research, Wolfson Laboratory for Molecular Pathology, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD.

TEXT BOOK EDITOR

Experienced text Book Editor of proven accomplishments to originate and develop lists in several disciplines, not exclusively scientific, at higher undergraduate level He or she will require an appreciation of the requirement of academics, students and markets within and without the United Kingdom He or she will report to the editorial director, and liaise with a small team of procurement editors, but will also enjoy considerable autonomy

Salary will be in excess of £6,000 and the company has a generous non-contributory pension scheme

Please write giving curriculum vitae to

Anthony Watkinson,
Senior Editor,
Academic Press Inc (London) Ltd ,
24–28 Oval Road,
London NW1 7DX.

8775(A)

UNIVERSITY OF MARBURG
DEPARTMENT OF ANATOMY AND CELL BIOLOGY
RESEARCH AND TEACHING ASSISTANTS

Two postgraduate research and teaching assistants are required to work on developmental aspects of the autonomic nervous system.

Experience in neurobiology, particularly in nerochemistry, cell culture and electron microscopy would be an advantage

Salary according to B A.T IIa (DM3.000,- to DM3 200,- per month).

The appointments may be taken up immediately

Applications (two copies) giving details of age, education, language abilities and experience together with the names and addresses of two referees should be sent to Professor K. Unsicker, Department of Anatomy and Cell Biology, Philipps-University, D-3550 Marburg, West Germany

8736(A)

UNIVERSITY OF RHODESIA
SCHOOL OF AGRICULTURE
TECHNICIAN AGRICULTURAL ENGINEERING

Applications are invited for the post of Technician in the newly-established Department of Land Management, in the School of Agriculture at the University of Rhodesia.

Duties. The successful applicant will assist with the practical classes, research and development work in Agricultural Engineering and Farm Mechanisation. He will be responsible for running a workshop and will be required to do outdoor work with field machinery The Workshop is equipped with a lathe, gas-electric webbing equipment and woodwork machinery

Further particulars with regard to these duties may be obtained by writing to the Chief Technician, School of Agriculture, University of Rhodesia, PO Box MP 167, Mount Pleasant, Salisbury, Rhodesia

Qualifications: Applicants should have served an apprenticeship or had similar training in workshop practice including the care of machine tools,) N C. City and Guilds full technological certificate or similar qualification would be an advantage, as would previous experience in similar work, particularly in Africa.

Salary Scale (Approx Stg. equiv): echnician/Senior Technician. £3,880 y 207 to £4,915 (Scale Barrier) by 79 to £6,868 per annum Entry point according to qualifications and experience up to a maximum commencing salary of £4,915 per annum

Applications: Six copies giving full details of personal particulars, qualifications, experience and the names of three referees should be sent to the Assistant Registrar (Science), University of Rhodesia, PO Box MP 167, Mount Pleasant, Salisbury, Rhodesia.

Closing date for receipt of applications. **December 31, 1978.**

British subjects considering applying for posts in Rhodesia are urged to consult the Foreign and Commonwealth Office (telephone 01-233 8777) their nearest British Consular Office.

8734(A)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF GEOLOGICAL SCIENCES
RESEARCH ASSOCIATE IN CRUSTAL SEISMOLOGY

The Associate will be concerned with running a network of temporary recording stations in the German Rheinish Massif for the period April-June, 1979 and analysing the data obtained, using the information from a concurrent explosion experiment to give a good control on crustal structure Applicants should have a good honours degree in geophysics, geology or physics with preferably some experience of seismological recording equipment. The appointment is tenable until June 30, 1981. Salary will be in the range £3,384 to £4,882 (under review), plus superannuation Starting salary will not exceed £3,883.

Further particulars are available from the Assistant Registrar, Science and Engineering, University of Birmingham, P O Box 363, Birmingham B15 2TT, to whom applications (three copies) including full curriculum vitae and naming three referees should be sent by Monday, January 1, 1979

Please quote ref· NGL 8737(A)

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)
RESEARCH TECHNICIAN

Required in the Medical Unit to assist in M R C. supported project on hypertension.

The post is for a maximum of three years Previous laboratory experience is expected and experience in steroid immunoassay and/or caecholamine assay would be an advantage. Candidates should have H.N C., M I Bol , B Sc. or equivalent

Initial salary £3,261 plus £354 London Allowance per annum

Enquiries to Dr H. L J. Makin (01-247 0644, ext. 71). Applications to the Secretary, The London Hospital Medical College, Turner Street, London E1 2AD, quoting ref RTMU/12/78

8739(A)

THE POLYTECHNIC OF CENTRAL LONDON
SCHOOL, OF ENVIRONMENT
RESEARCH ASSISTANT

in the Environmental Science Group tenable initially for ONE year, with the possibility of a further year. The successful candidate will study the utilisation of solar radiation for domestic space and water heating, and will concentrate on practical studies aimed at maximising the collection efficiency Applicants should have a degree in Physics

Salary £3,087 to £3,174 including £474 London Allowance

Details and application form from the Establishment Officer, P C L , 300 Regent Street, London WIR 8AL (01-580 2020 ext 212)

8731(A)

We have an immediate vacancy in the

London office for a

PHYSICAL SCIENTIST

preferably one who has some postdoctoral experience. He or she would be engaged largely in the assessment of scientific manuscripts submitted for publication but would probably also help with other sections of the journal. Experience in the earth, ocean or atmospheric sciences would be an advantage.

Write to:

The Editor
NATURE
MACMILLAN JOURNALS LIMITED
4 Little Essex Street, London WC2R 3LF

enclosing a curriculum vitae and any other material you consider relevant.

8661(A)

RESEARCH BIOCHEMIST REQUIRED

to work on a two year project on "Drugs and Breast Feeding". It is proposed to study the effect of prolactin stimulation on human lactation and transfer of several drugs into breast milk. The project is being supported by Birthright.

Applicants with a Ph D. would have an advantage. The salary £5,284 for the first year, and will be £5,508 in the second year. Applications to Dr David Harvey, Senior Lecturer in Paediatrics, Institute of Obstetrics and Gynaecology, Queen Charlotte's Maternity Hospital, Goldhawk Road, London W6 0XG.

8728(A)

IMPERIAL COLLEGE LONDON
Biochemist/Neurochemist

A Scientific Research Assistant with 2 or 3 years postdoctoral research experience is required for M.R C funded projects on cellular biochemical aspects of the organisation and function of neurotransmitter systems at synapses in the mammalian C N S A background in neurochemistry is not a strict requirement, but experience with incubating tissues, subcellular fractionation or affinity chromatography would be advantageous. The successful candidate would join a team of ten working on closely related topics

The post will be available from January 1, 1979 and would be renewable on an annual basis for at least 5 years

Salary would be commensurate with experience. Applicants should send curriculum vitae and the names of two referees to Dr H F Bradford, Department of Biochemistry, Imperial College, London SW7 2AZ,

8735(A)

HILL FARMING RESEARCH ORGANISATION
ANIMAL PRODUCTION AND NUTRITION DEPARTMENT

A vacancy exists for a Senior Scientific Officer/Principal Scientific Officer to work on aspects of body composition in the sheep An interest in intake measurement in grazing ruminants would be an advantage.

Qualifications a first or upper second class Honours degree or equivalent in a relevant branch of animal science, plus at least four years' relevant post-qualifying experience

Salary scale. S S O £5,154 to £6,898. P S O. £6,609 to £8,461

Non-contributory Superannuation Scheme

Application forms and further particulars may be obtained from the Secretary, H F R O , Bush Estate, Penicuik Midlothian EH26 0PY, to whom they should be returned not later than December 29, 1978. Please quote Ref. A/6/234

8705(A)

UNIVERSITY OF EXETER
DEPARTMENT OF BIOLOGICAL SCIENCES
DEMONSTRATOR IN FRESHWATER BIOLOGY

Applications are invited for a Demonstratorship in Freshwater Biology tenable from April 25, 1979 Applicants should have a good honours degree in Zoology, Biology or Ecology, and should also have, or be completing, a Ph D in some aspect of Freshwater Biology. The appointment is primarily zoological Some knowledge of freshwater plants and experience with freshwater fish would be an advantage. Duties will include research and teaching. In addition to assisting with the freshwater biology course the person appointed will be expected to demonstrate other zoological and ecological courses and to assist with field courses.

The salary range is £3,384, £3,634, £3,883, £4,133 per annum plus U S S membership. The post will be tenable for two years in the first instance, with a possible extension of a further two years. Applicants not having a Ph.D degree will be expected to register for such a degree. It will be tenable for two years for holders of a Ph.D. degree and the salary will be on the two top points of the range

Further particulars may be obtained from Miss Doreen B Watson, University of Exeter to whom applications (five copies) with the names of two referees should be sent by January 8, 1979 Interviews in connection with this post will be held on January 25, 1979 Please quote reference No. 7152

8757(A)

GAUBIUS INSTITUTE TNO
Leiden, The Netherlands
RESEARCH BIOCHEMIST *or* BIOLOGIST

The Gaubius Institute of the Health Research Organisation TNO has as its main assignment to investigate the pathogenesis of arterio-sclerosis Fields of research include: pathophysiology of the arterial wall, fibrinolysis and lipid metabolism.

VACANCY: Postgraduate research worker with experience in lipoprotein-leukocyte interactions and/or hormone receptor studies on circulating mammalian cells

DUTIES: In cooperation with the Leiden University Hospital medical staff and the Gaubius Institute lipid research team to initiate and conduct investigations on disorders in the control of lipoprotein metabolism as appearing in circulating cells.

TENURE: Four years (extension not excluded).

SALARY D.FL.49,000 to D.FL.57,000 per annum, dependent on qualifications and age

ENQUIRES: Dr L W Hessel, Gaubius Institute TNO (Tel: Leiden 071-131345).

APPLICATIONS: Preferably before December 20, 1978 and including curriculum vitae and names of two professional referees to:

The Director,
Gaubius Institute TNO,
Herenstraat 5 d,
Leiden,
The Netherlands 8700(A)

OXFORD UNIVERSITY
PHYSICAL CHEMISTRY LABORATORY
TEACHING LABORATORY OFFICER

The Department intends to appoint a Teaching Laboratory Officer to develop new and maintain existing experiments for the undergraduate course Candidates should be skilled in teaching practical physical chemistry and have some knowledge of electronics and computing, a part-time association with a research group is not excluded The appointment is on the scale RSIA, from £3,883 to £6,555 with U.S S Further particulars from Professor J S Rowlinson, Physical Chemistry Laboratory, South Parks Road, Oxford OX1 3QZ, to whom applications should be sent by February 17, 1979. 8760(A)

UNIVERSITY OF NOTTINGHAM
School of Agriculture
SUTTON BONINGTON LOUGHBOROUGH
DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL STUDIES
TECHNICIAN GRADE 3

required for a research project on maternal-fetal relationships during early pregnancy in the cow Techniques involved include tissue culture and electron microscopy Previous experience of at least one of these would be an advantage

The appointment is for 18 months, commencing February 1, 1978 Salary on scale £2,688 to £3,060 per annum (under review) Minimum qualifications O N.C , O.N.D or other recognised equivalent.

Write to the Secretary at the School for an application form and further details quoting Post Ref. 78/30

8759(A)

NATURAL ENVIRONMENT RESEARCH COUNCIL
UNIT OF INVERTEBRATE VIROLOGY
SCIENTIFIC OFFICER

A vacancy exists for a Scientific Officer to work on viruses of wild birds, in particular pigeons, starlings and gulls. Initially, the work will involve isolation of viruses in cell cultures and experimental animals, also to provide criteria for the recognition and monitoring of these viruses in the field

Qualifications: Applicants should have a degree or H N C in a biological subject.

Previous experience of virology and cell culture techniques would be an advantage as would the possession of a driving licence

Salary Salary is on the scale £2,839 to £4,415 per annum for 37½-hour week, starting point dependent on qualifications and experience

Further details and application form can be obtained from the Laboratory Superintendent, Unit of Invertebrate Virology, 5 South Parks Road, Oxford OX1 3UB 8681(A)

POSITIONS WANTED

GEOLOGIST, Ph D January 1979 Spec sedimentology, inorg geochem. Long exp teaching, uranium prosp , X-ray diffract , X-ray fluores AAS DTA, fluorimetry Shipboard and overseas exp Participation in inter sci proj Organisation talent Publ Perfect English and German 39 years, single. Europe, Australia Box No 8564, 'Nature', 3 Dyers Buildings, Holborn, London EC1N 2NR

8683(B)

ASSISTANTSHIPS

IMPERIAL COLLEGE
DEPARTMENT OF ZOOLOGY

Applications are invited for a postdoctoral Research Assistantship tenable at the College Field Station for work for three years on a Medical Research Council sponsored project on the sexual development of malarial parasites The successful applicant will be expected to develop new methods for the culture and purification of the parasite, and to study the biochemistry/physiology of the parasite plasmalemma Experience in the fields of cell fractionation of membrane chemistry would be an advantage

Salary in the range £3,883 to £4,631 on the Research and Analogous Staff scale, plus U S S

Applications with the names of two referees in writing by December 15, to Dr R E Sinden, Imperial College Field Station, Ashurst Lodge, Ascot, Berks. 8604(P)

UNIVERSITY OF BRISTOL
DEPARTMENT OF GEOLOGY
POSTDOCTORAL ASSISTANT: DEEP GEOLOGY, S. ENGLAND

Applications are invited for a (N E R C -funded) postdoctoral assistantship, the successful applicant to take up duties as soon as possible. The appointee will be a member of a team commissioned for three years to study the deep geology of southern England in collaboration with the Institute of Geological Sciences and Imperial College London. The Bristol-based members of the group will be concerned with the western part of the Variscan front between the Mendips and the Reading/Newbury magnetic anomaly. Applicants should have a strong background and training in geophysics and have a particular knowledge of geology Salary within the range of £3,883 to £5,129 (scale under review) Further information can be obtained from Professor D L Dineley, Department of Geology, University of Bristol, Queen's Building, University Walk, Bristol BS8 1TR, to whom applications including curriculum vitae and names of two referees should be sent Closing date for applications December 30, 1978

8692(P)

UNIVERSITY OF DURHAM
POSTDOCTORAL RESEARCH ASSISTANT IN PHYSICS

Applications are invited for an S R C Research Assistantship tenable for three years from early 1979 in a small group studying magnetic domains and crystal lattice defects by X-ray topographic techniques, under the direction of Dr B K Tanner The Research Assistant will be responsible for the construction and testing at overseas establishments of a video system for direct display of X-ray topographs due to be installed in 1980 on the Synchrotron Radiation Source under construction at Daresbury Laboratory

Initial salary in the range £3,883 to £4,382 on National Research Range 1A, plus superannuation

Applications (three copies) naming three referees should be sent by January 1, 1979 to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained.

8686(P)

FOR SALE

FOR SALE

E M 6G ELECTRON MICROSCOPE Fully maintained and in full working order Offers to. Dr R. H Cousens, Gillette Research Laboratory, 454 Basingstoke Road, Reading RG2 0QE Tel 0734 85222 8727(L)

FELLOWSHIPS

INVERTEBRATE NEUROBIOLOGY
RESEARCH FELLOWSHIP

available for 2–3 years to study molecular mechanisms involved in control of electrical properties of molluscan neurones Experience with intracellular recording (preferably in *Aplysia*) required

CV and three letters of recommendation to. Dr I Levitan, Friedrich Miescher-Institut, P O Box 273, Ch-4002 Basel, Switzerland 8643(E)

UNIVERSITY OF EDINBURGH
DEPARTMENT OF VETERINARY PHYSIOLOGY
POSTDOCTORAL RESEARCH FELLOWSHIP
(Scale IA: £3,883 to £6,555 per annum)

Applications are invited for the above post, which is supported by an M R C Programme Grant to Dr A G. Brown, tenable for five years from January 1, 1979 The Fellow will join a team studying the electrophysiology, ultrastructure and quantative morphology of identified spinal cord neurones The Fellow will be responsible for computer graphics analysis of neuronal structure. He or she will need to be conversant with computer technology, graphics systems, programming and electronics An interest or experience in neurobiology would be desirable A Joyce-Loebl MAGISCAN image analysis system containing a NOVA 3/12 computer will be available

Applications, including the names and addresses of two referees, should be sent as soon as possible to Dr A. G Brown, Department of Veterinary Physiology, University of Edinburgh, Summerhall, Edinburgh EH9 1QH Please quote Reference 5070.

8640(E)

EMBO
European Molecular Biology Organisation
LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY
SPRING 1979 AWARDS
Next deadline: February 20, 1979

The long term fellowships will usually be for a period of up to one year but applications for a renewal for a second year are considered To be eligible a candidate must hold a doctors degree. Preference will be given to European and Israeli applicants wishing to work within Europe or Israel, EMBO long term fellowships are NOT, however, awarded for exchanges between laboratories within any one country Applications for fellowships to be held outside Europe and Israel are considered but they have a lower priority, as do applications from non-European scientists wishing to work in Europe or Israel

The deadline for applications for the 60 fellowships to be awarded in April 1979 is February 20, 1979 Because the selection procedure involves an interview this deadline should be respected

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organisation, 69 Heidelberg 1, Postfach 1022 40, F R Germany 8682(E)

THE UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF ANATOMY
POSTDOCTORAL RESEARCH FELLOWSHIP

A postdoctoral biochemist is required to join a multidisciplinary team studying aspects of growth and differentiation The successful candidate will be responsible for a new M.R C-funded project involving the use of monoclonal antisera to study protein polymorphism in muscle Technical support will be available. The post is for three years beginning as soon as possible and the salary will be on the Research Fellow 1A scale £3,883 to £6 555 plus superannuation

Applications (three copies), enclosing a curriculum vitae, details of research experience and the names of three referees, should be sent to the Assistan Registrar, Faculty of Medicine, University of Birmingham, Birmingham B15 2TJ, from whom further information may be obtained Closing date January 5, 1979 Please quote reference RF/ANT/478 8753(E)

FELLOWSHIPS (continued)

MASSEY UNIVERSITY

Palmerston North, New Zealand

POSTDOCTORAL FELLOWSHIPS

Applications are invited for Postdoctoral Fellowships tenable in any Department of the University.

The academic activities of the University are grouped under the following faculties and schools.

Opportunities for study and research are available in the following fields.

AGRICULTURAL AND HORTICULTURAL SCIENCE

Agricultural Economics and Marketing
Farm Management
Agricultural Engineering
Agronomy
Animal Science (Dairy, Sheep, Beef, Pigs)
Horticultural Science and Plant Health
Poultry Science
Soil Science

BUSINESS ADMINISTRATION

Agricultural Business
Business Studies
Data Processing
Economics
Financial Management and Accounting
Marketing
Personnel Administration

FOOD SCIENCE AND BIOTECHNOLOGY

Food Technology
Biotechnology (i.e. processing of Biological Materials)
Industrial Management and Engineering
Industrial Management and Industrial Mathematics

HUMANITIES

English
History
French
German
Philosophy

SCIENCE

Biochemistry
Biophysics
Botany
Chemistry
Genetics
Microbiology
Zoology

SOCIAL SCIENCE

Applications from scholars in any of the usual Social Science disciplines will be welcomed, but particular preference may be given to those interested in Educational Sociology, Human Geography and/or Planning, Mathematics, Social and Applied Psychology, and Economics.

VETERINARY SCIENCE

Physiology and Anatomy
Veterinary Pathology and Public Health
Clinical Sciences

Information concerning the research activities of the departments associated with these groups are given in the University Calendar. Intending applicants are advised to write to the Head of the appropriate department in the University in the course of preparing an application.

The Fellowship will be tenable for one year, with possible extensions, and carries an emolument of NZ$8,500. Travelling expenses up to NZ$1,500 may be provided.

Further details of the awards and of the University, together with conditions of appointment and information to be supplied by applicants, may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close on **January 19, 1979.**

8721(E)

Mental Health Foundation

FELLOWSHIPS

Applications are invited for **Mental Health Foundation Fellowships** from suitably qualified persons wishing to pursue **Full-time Research Work** bearing on problems of mental health, mental illness or mental handicap, whether in clinical psychiatry or in one of its supporting sciences.

Both **Junior and Senior Fellowships** are offered in the salary ranges of Junior/Senior Lecturer, Junior/Senior Registrar, according to seniority and experience, plus superannuation. However, Senior Fellowships are only awarded under exceptional circumstances, e.g., as a temporary measure prior to the Fellow taking up a permanent career post. The appointments, which are also open to candidates from overseas, will be for up to **three years** in the first instance and in exceptional circumstances may be extended to **five years**. Interviews will be held in May in London and the Fellowships will be taken up between the following July 1 and March 31. Overseas candidates, if shortlisted, must attend for interview at their own expense.

Closing date: March 1.

Application forms and further information may be obtained from The Secretary (N), The Research Committee, Mental Health Foundation, 8 Wimpole Street, London W1M 8HY.

8669(E)

UNIVERSITY OF DUNDEE

Candidates with appropriate postgraduate qualifications in Biochemistry are invited to apply for appointment to a

POST DOCTORAL RESEARCH FELLOWSHIP

to work with Dr J. L. Young (Department of Obstetrics and Gynaecology) and Dr D. A. Stansfield (Department of Biochemistry) on a M.R.C. funded study of the hormone receptor/adenylate cyclase complex of the corpus luteum.

The appointee will be based in the Department of Obstetrics and Gynaecology at Ninewells Hospital and Medical School, Dundee but close co-operation with the Department of Biochemistry will be maintained.

The appointment is available now for a period up to November 30, 1981. The initial salary will be dependent of qualifications and experience but within the range £3,883 to £5,555

Applications, quoting Reference EST/69/78J and containing the names of two referees, should be lodged as soon as possible with The Secretary, The University, Dundee DD1 4HN.

8697(E)

1979 BEIT MEMORIAL FELLOWSHIPS FOR MEDICAL RESEARCH

Notice is hereby given that an election of Junior Beit Fellows to begin work on October 1, 1979 will take place in May 1979. Junior Fellowships carry an initial value of £3,883 to £4,631 plus £450 London Allowance, plus yearly increments for three years. There will be the usual university superannuation contributions and benefits. Candidates must have taken a degree in a faculty of a university approved by the Trustees in Her Majesty's Dominions, Protectorates and Mandated Territories, India, Pakistan, the Republic of Ireland and the United Kingdom, or a medical diploma registrable in the U.K. Elections to Junior Fellowships are rarely made above the age of 35 years. Applications must be received from candidates not later than March 23, 1979. Candidates must submit evidence where they propose to work; which must be in Great Britain or Ireland.

Forms of application and all information may be obtained from Professor W. G. Spector (Secretary), Pathology Department, St Bartholomew's Hospital, London EC1A 7BE. For overseas candidates forms of application may be obtained from, The Secretary, South African Medical Council, P.O. Box 205, Pretoria, South Africa, The Ministry of Health, The Government of India, New Delhi, India, The Secretary, Department of Education and Science, P.O. Box 826, Canberra City, A.C.T. 2601, Australia; The Department of Health, Wellington, New Zealand; and, The Canadian Medical Association, 1867, Alta Vista Drive, Ottawa 8, Ontario, Canada

8668(E)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF GEOLOGY AND MINERALOGY

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited from suitably qualified organic geochemists or clay mineralogists to work with Dr M. J. Pearson on the correlation of organic matter and clay mineral diagenesis in sediments from the North Sea Basin. The project involves the study of kerogen maturation and hydrocarbon generation during burial in relation to clay mineral transformations as a response to temperature increase and ion concentration in

The post is tenable for three years and is supported by the Natural En-
Interstitial water.
vironment Research Council.

Salary £3,883 to £4,382 per annum on Research and Analogous Staff Range 1A Scale.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (two copies) should be lodged by December 29, 1978

8730(E)

UNIVERSITY OF LIVERPOOL

RESEARCH FELLOWSHIPS

The University is offering a small number of University Research Fellowships to be held in any Department of the University from October 1, 1979 for periods of up to three years. Salary will be in the range £3,883 to £7,754 p.a. (under review) depending on age and experience. Further particulars and application forms may be obtained from the Registrar, The University, P.O. Box 147, Liverpool L69 3BX, by whom completed forms should be received not later than January 31, 1979. Quote Ref: RV/998/N

8672(E)

COURSES

European Molecular Biology Organisation

EMBO PRACTICAL COURSE INTRACELLULAR Ca^{2+} MONITORING TECHNIQUES

Gif sur Yvette, France

June 11–21, 1979

The course is intended for workers who will find intracellular Ca^{2+} monitoring techniques of immediate use in their research programmes. Participants will be trained in the use of the most advanced techniques for monitoring free Ca^{2+} changes in living cells; i.e. photoproteins, metallochromic indicator dyes, and Ca^{2+} sensitive microelectrodes. Most of their time will be spent in the laboratory, working in groups of three, applying these techniques to nerve and muscle cells. There will also be lectures by the teaching staff, and seminars by invited speakers.

Instruction will be in English. In addition to the course organisers and the staff at Gif sur Yvette (G. Baux, R. T. Kado, T. Shimahara, M. Thieffry) teachers will include A. K. Campbell, M. V. Thomas, R. Rudel and M. Oehme.

The course is for a maximum of 15 participants. Applicants should preferably have some experience of electrophysiological techniques, and give a convincing reason for taking the course. Applicants should also include a brief curriculum vitae and be sent to Dr J. Stinnakre by February 15, 1979.

Acceptance for the course will be notified to applicants by April 1, 1979. Some grants will be available to partially defray living and travel expenses. Accommodation will be arranged by the course organisers.

Course organisers:

J. Stinnakre, Cours EMBO, Laboratoire de Neurobiologie cellulaire de C.N.R.S., F 91190 Gif sur Yvette, France.

I. Parker, Biophysics Department, University College London, London WC1E 6BT, U.K.

8680(D)

COURSES continued

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

LABORATORY COURSE ON RECOMBINANT DNA

10-27 JULY 1979

INSTITUT DE RECHERCHE EN BIOLOGIE MOLECULAIRE
2 Place Jussieu, Paris, France

The experimental programme will include·

In vitro recombination of DNA segments with plasmid and viral vectors

In vitro packaging of bacteripohage lambda DNA, Cosmids

Cloning in *E. coli, B. subtilis* and *S. cerevisiae*

Characterization of cloned recombinant DNA by restriction mapping, hybridization and sequencing

The teaching staff will include:

M L BACH (Strasbourg)
G. CUNY (Paris)
J DOLY (Paris)
S D. EHRLICH (Paris)
C GAILLARD (Paris)
A. GOZE (Paris)
A. HINNEN (Basel)
B. HOHN (Basel)
A. KLIER (Paris)
M MEUNIER (Paris)
B NIAUDET (Paris)
V SGARAMELLA (Milan)
F STRAUSS (Paris)

Introductory and research seminars will be given by members of the teaching staff and by invited speakers

Attendance at the Course will be limited to 20 participants Preference will be given to applicants from Europe

The registration fee will be 500 FF The participants will normally be expected to pay their own travel, accommodation and living expenses

Applications with a short curriculum vitae and any information which might help in the evaluation of the application should reach by March 31, 1979, Dr Giorgio Bernardi at the above address

8756(D)

GRANTS

Mental Health Foundation

RESEARCH GRANTS

The Research Committee of the Foundation exists to foster and support research in all fields bearing on mental health, mental illness and disorders of mental development

The Committee meets twice a year to award RESEARCH GRANTS TO PROVIDE SCIENTIFIC ASSISTANCE, EXPENSES or EQUIPMENT for research workers

For Grant application forms and further details apply in writing to

The Honorary Secretary (N),
The Research Committee,
Mental Health Foundation,
8 Wimpole Street,
London W1M 8HY

8670(H)

STUDENTSHIPS

STRATHCLYDE REGIONAL COUNCIL

GLASGOW COLLEGE OF TECHNOLOGY

DEPARTMENT OF BIOLOGICAL SCIENCES

SRC STUDENTSHIP in Biochemistry or related subject areas to work on the "Sodium Pump" in essential hypertension Write or phone for further particulars to Dr T Duffy, Department of Biological Sciences, Glasgow College of Technology, Cowcaddens Road, Glasgow G4 0BA. Tel· 041-332-7090, Ext 283 or 340 not later than December 15, 1978.

E. Miller, Director of Education.

8698(F)

INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE (INSERM)
EUROPEAN MOLECULAR BIOLOGY ORGANIZATION (EMBO)

MOLECULAR AND CELLULAR ASPECTS OF OBESITY

AUGUST 27-29, 1979
NICE, FRANCE

The workshop is intended to bring together scientists actively working on different aspects of this topic The number of participants is limited to about 60

Sessions

1—Differentiation in culture of mammalian cells into adipocytes

2—Control of fatty acid synthesis and esterification in hepatocytes and adipocytes

3—Control of lipolytic activities in adipocytes

Speakers (preliminary list)

G AILHAUD (Nice)
A W ALBERTS (Rahway)
P. BELFRAGE (Lund)
R M BELL (Durham)
P BJORNTORP (Goteborg)
D N BRINDLEY (Nottingham)
R M. DENTON (Bristol)
H GREEN (Cambridge-USA)
D LANE (Baltimore)
T OLIVECRONA (Umea)
D A K RONCARI (Toronto)
O ROSEN (New York)
T RUSSELL (Miami)
P. THOMOPOULOS (Paris)

Communication and Poster Sessions

An invitation to submit communications or posters is extended to all prospective participants

Applicants

Applicants may support their application by including one or more of the following a list of recent publications, an abstract for a communication or for a poster, a brief summary of the applicant's interests in this area

Applications should be sent before April 15, 1979, to

Dr G AILHAUD
CENTRE DE BIOCHIMIE
FACULTE DES SCIENCES
Parc Valrose
06034 NICE (France)

Applicants will be notified of acceptance by May 31, 1979

Registration

There will be no registration fee The organisation will cover accommodations and full board Sessions, meals and lodging will be at College International de Sophia-Antipolis (Nice) 8770 (D)

STUDENTSHIPS

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY
University of London
S.R.C CASE STUDENTSHIP

Cost and Energy Evaluation of Mineral Extraction Processes

A Science Research Council C A S E Studentship is offered in association with the Department of Industry, Warren Spring Laboratory for a study to develop a methodology of cost and energy evaluation of mineral extraction processes The methodology will be developed with particular reference to the extraction of alumina from indigenous U K resources of aluminiferous materials

Candidates from any background will be considered, but the study would be of particular interest to those with experience in mineral technology, extraction metallurgy, chemical engineering or chemistry

Applicants should have, or expect to obtain a 1st or upper 2nd class honours degree

Applicants should send details of their qualifications plus the names and addresses of two referees, to Professor R N Pryor, Head of Department, Department of Mineral Resources Engineering, Imperial College of Science and Technology, Prince Consort Road, London SW7 2BP. 8773(F)

ATHROFA GOGLEDD-DD CYMR
THE NORTH E WALES
INSTITUTE OF HIGHER
EDUCATION

RESEARCH STUDENTSHI IN DAIRY CHEMISTRY

The Joint Committee of the M
Marketing Board and the Dairy Tr
Federation are sponsoring a three-y
studentship for research on flav
deterioration in pasteurised m
supervised by Dr J C Allen T
grant will be at S R.C rates, p
fees, and the work is expected
lead to a Ph D

Applicants should be good hono
graduates in Chemistry, Biochemis
or Food Science

Applications by letter (no forms)
The Institute Registrar, North
Wales Institute of Higher Educati
Kelsterton College, Connah's Qu
Deeside, Clwyd, Tel Deeside 8175
Closing date for receipt of applicatio
December 15, 1978. 8754(F

CONFERENCES

NATO ADVANCED STUDY INSTITUTE

Semiclassical Methods in Scatteri
and Spectroscopy, Cambridge, U
September 9 to 22, 1979

Information and application for
may be obtained from Dr M S Chi
Theoretical Chemistry Department,
South Parks Road, Oxford OX1 3T
U.K. 8752(C

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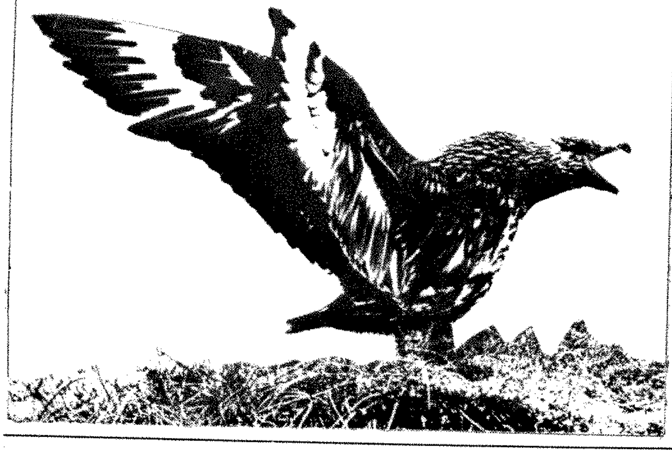

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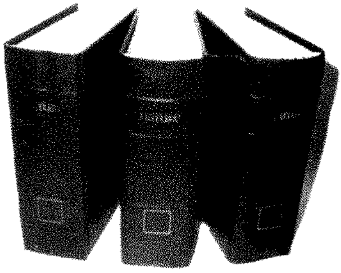

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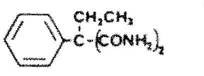
Biochemical Tools

16 MAY 1979

Anticonvulsant drugs - metabolites and analytical standards

Primidone:

2-Ethyl-2-phenylmalonamide (1, PEMA) is a metabolite[1]; **4-methylprimidone (2)** and **2-ethyl-2-(*p*-tolyl)malonamide (3, MPEMA)** are reference standards.



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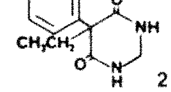

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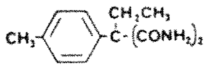

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1) *J. Chromatogr. Sci.*, **145**, 97 (1978)

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19,496-4	2-Ethyl-2-(*p*-tolyl)malonamide	250mg $6.05 1g $17.60

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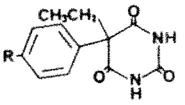

4, R OH
5, R CH_3

2) *Eur. J. Clin. Pharmacol.*, **9**, 161 (1975); *J. Pharmacol. Exp. Ther.*, **116**, 326 (1956). 3) *J. Chromatogr. Sci.*, **12**, 256 (1974); *Res. Commun. Chem. Pathol. Pharmacol.*, **13**, 41 (1976)

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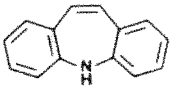

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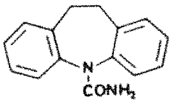

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4) *Clin. Chim. Acta*, **43**, 69 (1973); *J. Pharm. Pharmacol.*, **25**, 340 (1973); *Anal. Chem.*, **45**, 1846 (1973). 5) *Clin. Pharmacol. Ther.*, **14**, 827 (1973); *J. Chromatogr.*, **145**, 97 (1978).

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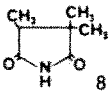
α,α-Dimethyl-β-methylsuccinimide (8)[6] and **α-methyl-α-propylsuccinimide (9)**[7] are reference standards.

Methsuximide:

α-Methyl-α-phenylsuccinimide (10) is a major metabolite.[8]



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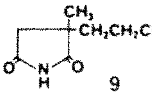

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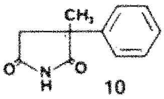

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6) *J. Clin. Pharmacol.*, **9**, 393 (1969); *Clin. Chim. Acta*, **33**, 87 (1971); *ibid.*, **60**, 285 (1975); *J. Chromatogr. Sci.*, **12**, 630 (1974). 7) *J. Anal. Toxicol.*, **2**, 39 (1978). 8) *J. Pharm. Sci.*, **62**, 1820 (1973); *Br. J. Pharmacol.*, **45**, 48 (1972)

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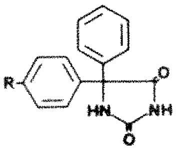
5-(*p*-Hydroxyphenyl)-5-phenylhydantoin (11, HPPH) is a major metabolite;[9] **5-(*p*-methylphenyl)-5-phenylhydantoin (12, MPPH)** is a reference standard.[10]



11, R OH
12, R CH_3

9) *J. Chromatogr.*, **82**, 307 (1973); *Clin. Chem.*, **22**, 1672 (1976); *Clin. Pharmacol. Ther.*, **14**, 791 (1973). 10) *Clin. Chem.*, **19**, 590 (1973); *ibid.*, **21**, 751 (1975); *Clin. Chim. Acta*, **48**, 135 (1973); *ibid.*, **62**, 73 (1975); *J. Chromatogr. Sci.*, **12**, 256 (1974).

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Di-n-propylacetic acid (valproic acid):

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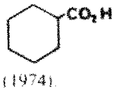

11) *Pharm. Weekbl.*, **109**, 1 (1974).

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And you can get rid of all those rabbits

The protocol is straightforward, and it includes suggested final assay procedures. So that's our message. Now add yours.

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Control mRNA Purified mRNA to check the biological activity of the system.

Salts and sterile deionized water Selected and prepared to minimize potential ionic or enzymatic inhibition of the system.

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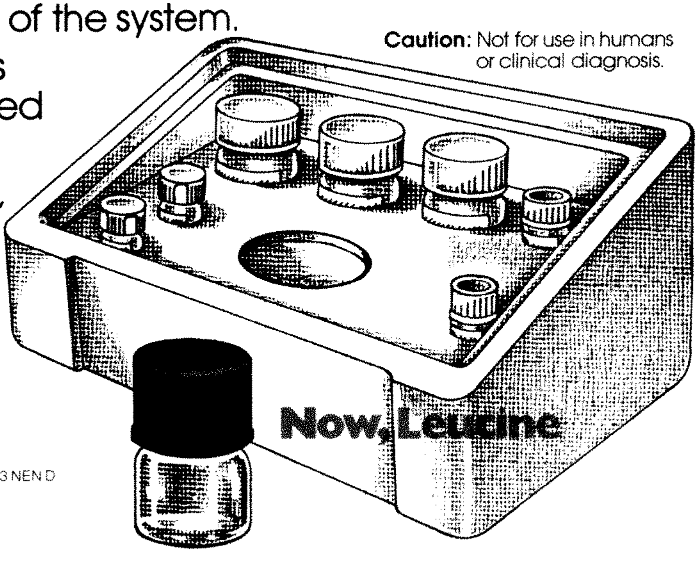

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Flint blade from Volgu, Saône-et-Loire, France, about 17,000 BC, length 28.5cm. ESR spectroscopy is now being used to study flint; see page 703. (Photo, Trustees of the British Museum.)

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Human rights in China

MANY Western scientists have experienced the excitement in the past few years of establishing or re-establishing ties with scientists from the People's Republic of China. The openness and frankness of these exchanges (particularly in comparison with the unreal nature of some exchanges with the Soviet Union) has surprised and pleased those involved. Even though there clearly have been and continue to be strong political winds blowing in China, it has been widely anticipated that China will continue to take a fuller place in the international scientific community. But many of the more thoughtful visitors have left wondering what the true price is that the people have to pay to live in modern China. Some inkling of this now emerges from a recent Amnesty International report 'Political Imprisonment in the People's Republic of China'.

The constitution guarantees a number of basic rights, such as 'freedom of speech, correspondence, the press assembly, association, procession, demonstration and the freedom to strike'. In addition citizens have the right to 'speak out freely, air their views fully, hold great debates and write big-character posters'. Furthermore the state 'applies the principle of "letting a hundred flowers blossom and a hundred schools of thought contend" so as to promote the development of the arts and sciences and bring about a flourishing socialist culture'. However, all cultural undertakings must 'serve the workers, peasants and soldiers and serve socialism', and the state 'suppresses all treasonable and counter-revolutionary activities, punishes all traitors and counter-revolutionaries, and punishes newborn bourgeois elements' and 'other bad elements'.

A continuous campaign of purification seems to go on against counter-revolutionary elements, who face a wide range of punishments from public criticism to execution for their crimes of announcing reactionary solutions or writing reactionary posters. Trials leave a lot to be desired; the main aim seems to be to extract a confession, and defence is a rare luxury. Indeed those who refuse to acknowledge their wrongdoing are subjected to much severer sentences. The number of counter-revolutionaries that courts have dealt with is difficult to estimate, but clearly runs into many tens of thousands. Chairman Mao himself once estimated the hardcore of reactionaries at two per cent of the whole population.

The Amnesty report is not cheerful reading; the crude ways in which those who do not follow the official line are humiliated, imprisoned or even exterminated cannot but worry all those who wish to establish the closer diplomatic, cultural and intellectual relations with China. And presumably for every one who runs foul of the judicial system for unacceptable views there are many who simply choose to remain silent. ☐

Research councils dislocated

ON December 18, Shirley Williams, Britain's Secretary of State for Education and Science will officially open the new premises of the Science Research Council and the Natural Environment Research Council at Swindon. This is the final step in a five-year process by which all but a handful of the staff of the two councils have been moved out of London. And the true dimensions of the inconvenience it will cause are now becoming all too clear.

For many of the staff, of course, the move to a more rural environment has had its attractions. Those whose work does not directly involve attending or servicing meetings or being on hand for the scientist who wishes to drop in may well feel that they have gained. But those whose effectiveness depends on easy contact with a wide variety of people have much more reason to complain. In the SRC, for instance, the Chairman's office stays in London, as do the directorates of marine technology and polymer engineering. But the Secretary to the Council is in Swindon, as is the Press Office. Both chairman and secretary are expecting to have to spend an average of a day and a half per week at the other end of the line.

At present SRC council and committee meetings are continuing to be held in London, scattered around the premises of various learned societies. This is hardly an ideal solution, but in the future, wear and tear on council staff may well lead to pressure for more meetings to be held in Swindon, entailing complex cross-country journeys for those who do not live near London, and presumably necessitating more overnight stays. Fine in some respects; if visiting academics and industrialists can be prevailed upon to give up an extra half day, the quality of committee work will probably rise. But it is every bit as likely that attendance at meetings will fall.

Life in Swindon will be fine for a majority of the councils' staff. But the more senior the person, the more inconvenient and tiring it will be. The net result could well be that it will become much more difficult to recruit to the top posts, and that committee members will become much less willing to give of their time. ☐

Crisis for sperm whales

The International Whaling Commission (IWC) is holding a special meeting in Japan next week. Two major tasks for the meeting will be to set catch limits for sperm whale stocks in the North Pacific and to review the status and catch limits of sperm whales in the Southern Hemisphere. John Beddington, lecturer in population biology at the University of York, explains why he thinks no more sperm whales should be caught in these areas.

AT ITS meeting in Cambridge this summer the scientific committee of the International Whaling Commission identified a number of worrying questions concerning the current state of the world's sperm whale stocks. These questions, which were the subject of further discussions when the committee reconvened in La Jolla in November, pose problems both for the whales and the IWC.

Since the 19th century the sperm whalers have concentrated their hunt on the older males, as there are sound economic reasons for preferring these larger animals. More recently it has been recognised that because the sperm whale is polygamous there is a clear opportunity of obtaining an increased yield from populations with a substantially disturbed sex ratio. However, there is a danger that if too many mature males are taken, pregnancy rates will fall as females fail to find mates. This danger is recognised in the current management model used by the IWC which demands that a reserve of socially mature males should be excluded from exploitation to ensure maximal pregnancy. Unfortunately, in two major sperm whale stocks there have been disturbing falls in the pregnancy rates of the female catch. These changes, in the Australian sector of the Southern Ocean and in the Western division of the North Pacific gave a clear indication that all was not well with these stocks.

This problem was illuminated by other scientific analysis by Beddington, Holt, and Fowler which showed that the way in which the IWC had estimated population abundance seriously masked the extent of the depletion of stocks following exploitation. The mechanism involved is simple. The main measure of population abundance used by the IWC is the catch per unit of fishing effort and the measure of effort that has been used was the number of catcher boat days. However, catching whales takes time and daily catch rates at the start of the fishery were limited not by the population abundance but by the time taken for catching whales. Accordingly as stocks declined there was no corresponding drop in catch rates and hence stocks were thought to be more abundant than was in fact the case.

When data for the Western North Pacific stock were reanalysed in the light of these observations it was found that in the period of the observed fall in pregnancy rates the mature male population had fallen below the reserve level and hence pregnancy rates would have been expected to fall. Similar analysis of the Australian stock was not carried out in Cambridge due to lack of time, but some members of the Scientific Committee were of the view that given the substantial fall in pregnancy rates, a similar effect was occurring and that to set a zero quota would be the appropriate response of the commission. In the event this view was not accepted and the quota for this division was reduced by only 25%. Unfortunately, subsequent analysis submitted to the Australian Whaling Enquiry by Kirkwood, Allen & Bannister vindicates this pessimistic view and indicates that here again mature males were reduced below their reserve level and that pregnancy rates would be expected to have fallen. The status of both these stocks gives much cause for concern and it should be noted that as comparable data on the pregnancy rates are not available for all stocks the problem may not be restricted to these areas alone.

A final problem highlighted by the sperm whale analysis concerns the inadequacies of the new management procedure used by the IWC to set quotas. This procedure provides for protection of stocks when they fall 10% below their maximum sustainable yield level. In both the cases considered above, strict interpretation of these rules would permit quotas to be taken—females from the Australian division of the Southern Ocean and both sexes from the Western North Pacific stock. However, simple extrapolation of the demographic statistics indicate that even in the absence of exploitation these stocks would continue to decline for decades. If subsequent analysis corroborates these findings the Whaling Commission will thus be faced with the difficult task of deciding whether to interpret its rules legalistically or to protect these whale stocks. □

Cornelia Durrant, Wildlife Campaigner at Friends of the Earth, UK, adds: At the end of last October it seemed that the UK government had taken some action to protect whales when it announced its proposals for the meeting of the Convention on the International Trade in Endangered Species (CITES) to be held next March. CITES aims to control international trade in wildlife and wildlife products. Those species which the 48 member nations agree are 'endangered' are listed on appendix I of the convention and trade in them is normally forbidden. Those which are considered 'threatened' are listed on appendix II. Trade in them is allowed but monitored.

The UK has been proposing a review of cetacea for next year's CITES meeting. It proposes that 11 species of dolphins and porpoises should be put on appendix I and that all cetacea not on appendix I should be listed on appendix II—including the sperm whale.

When this was announced it received a great deal of attention. So much so that the implications of the UK's other proposal to CITES were not fully explained. The convention requires all 'readily recognisable' parts and products of listed species to be controlled, but there is disagreement over what is recognisable, and many members control no products at all. Together with Switzerland and West Germany, the UK has prepared a list of parts and derivatives of several species of animal which it would like to see all parties control.

This list, called the 'minimum list' of parts and products, is surprising in many respects. Most surprising of all it does not include whale products because "whale meat, meat products and oil were not considered to be identifiable without the use of biochemical laboratory equipment". However, the UK has always identified whale products for Customs tariff purposes.

Consequently even if all cetaceans are put on the appendices to CITES only trade in live animals will be controlled—and this is only a few dolphins a year. The trade in their products will continue unchecked.

Recent data on the status of sperm whales shows several populations to be more than just 'threatened', in particular those in the Western division of the North Pacific and those in divisions five and seven of the Southern Hemisphere. Consequently there is a strong case for the UK proposing these populations of sperm whales for appendix I of CITES. If, however, the 'minimum list' is accepted in its present form this would be of little use. As it is clearly impossible to distinguish between the products of whales from different populations the only way to ensure that the endangered ones are not further depleted is to control the products of all sperm whales. □

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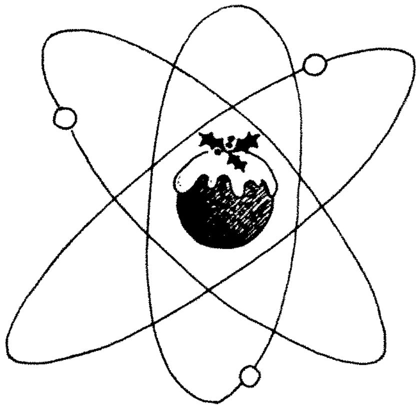
Surprise Christmas present for British science



An unexpected Christmas bonus for British science was revealed this week by the Secretary of State for Education and Science, Mrs Shirley Williams, who announced that the country's science budget was to be increased by more than £47 million over the next four years. More than 70% of this money is to go to the Science Research Council and the remainder is to be divided between the Agricultural Research Council, the Medical Research Council, and the Natural Environment Research Council. Only the Social Science Research Council budget remains static.

The budget increase represents a significant Cabinet victory for Mrs Williams who has been arguing strongly all year for increases in funds for basic scientific research—which, she said, had suffered too severely at the expense of applied research. Now the science budget is to be increased by £10 million in 1979-80; £10 million in 1980-81; £12 million in 1981-82 and £15 million in 1982-83. The move will increase next year's budget to £274 million of which £149.7 million will go to the SRC.

The increase reflected a change in world opinion which was now swinging back to a more favourable view of basic research, said Sir Frederick Stewart, chairman of the Advisory Board for the Research Councils, the body responsible for dispersing the science budget among the five research councils. He quoted recent developments in the United States and France where there had been increases in money for fundamental research.

Mrs Williams said Britain had insufficiently recognised the achievements of its basic science which was regarded highly elsewhere in the world. This extra cash would attempt to remedy this and would also be used to create new research positions for able young students who were now suffering severe problems in finding posts.

Predictably the happiest research council chairman was Professor Geoffrey Allen of the SRC who described himself as "delighted" with his cash allocation. Last month he announced at the publication of his council's annual report that the SRC needed only £20-£30 million to carry out a full but realistic programme of research over the next four years. In fact, the budget increase means he will now have slightly more than £30 million for this programme, of which £4 million will become available next year.

Research allocations

	1979-80	1980-81	1981-82	1982-83
	£m	%	%	%
ARC	24.7	+1.9	+1.1	+1.0
MRC	46.5	+1.6	+0.9	+1.0
NERC	31.1	+2.5	+1.5	+1.0
SRC	149.7	−1.0	−0.5	+1.0
SSRC	14.8	0.0	0.0	0.0
BM(NH)	4.6	+0.5	+0.5	+1.0
Royal Soc	2.6	+1.0	+1.0	+1.0
	274.0			

Initially much of this money will go to restoring the SRC's construction programme to previous levels, including the speedy completion of synchrotron radiation, laser and electron beam lithography facilities at the SRC's laboratories, and also in setting up research programmes in computer and microprocessor technology. It is also hoped that Britain will get a new space programme involving participation in the United States' MMS re-usable satellite project.

As for the other research councils, it is likely that the extra cash will be used on projects including increased research into cancer and its treatment and also to set up a new unit of environmental epidemiology for the Medical Research Council; to improve geological work in deep drilling and mapping for the Natural Environment Research Council; and to support work for the Agricultural Council which would involve developing crops which could fix their own atmospheric nitrogen.

Robin McKie

Blind man's buff at GMAG

On 31 December, the two-year term of office for members of the UK's Genetic Manipulation Advisory Group (GMAG) will come to an end; some members will be leaving and new people appointed in their place. But the criteria used to decide who will stay on and who will leave are a deepening mystery.

The only official information that has been made public about the changes is that Sir William Henderson (until recently Secretary to the Agricultural Research Council) will succeed Sir Gordon Wolstenholme as chairman. A spokesman at the Department of Education and Science told *Nature* last week that in the interest of "balance and continuity", the four sectors represented on GMAG (science, industry, the unions and the public) would continue to be represented and that not all the members of the existing GMAG would be asked to stand down. According to John Morris, GMAG secretary at the Medical Research Council, however, that does not mean that "some members who may wish to stay on will not be sacked".

At least one member of the group, Dr J. R. Ravetz, a public interest representative, can testify to this. Three months ago all members of GMAG were sent a letter asking if they wished to continue. Dr Ravetz, a reader in the history and philosophy of science at the University of Leeds, had played a particularly active role in GMAG and did not wish to leave, but was sent a letter asking him to stand down. He was given no reason other than that new appointments "are necessary to ensure the balance and continuity of the group".

However, two of the three other public interest representatives have indicated that they wish to stand down for personal reasons. Continuity in the public interest sector, Ravetz argues, is therefore being carried on the shoulders of the only remaining public representative, John Maddox. Mr Maddox was unaware that two of the public representatives were leaving voluntarily until contacted by *Nature* last week.

What then are the criteria used to decide which representatives should be removed, and who is responsible for making these changes? For the union and industry representatives, the situation is clear. They are nominated or recommended to stand down by their respective organisations and these recommendations are generally accepted by the DES. The Trades Union Congress (TUC) has asked to be represented on the new GMAG by its four original representatives and by one new member. The four serving TUC members have been reappointed by the DES, but there is no word yet about

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the request for a fifth representative. The Confederation of British Industry has requested that its representative Mr John Gilby, be renominated and is awaiting a reply.

The situation of the science and public interest representatives is, however, considerably less clear. They are directly appointed by the DES but there appears to be no set procedure governing these appointments beyond "usefulness to the group". Ravetz has certainly been useful, but he has also not shrunk from expressing his opinions, and he may have been seen by some close to GMAG to be 'rocking the boat'.

According to one DES spokesman, the public interest representatives are the ones most likely to change "because the scientific specialists are a small group to choose from", but he expects that the overall "mixture of representatives" will remain the same—except that possibly a lawyer will be included on GMAG for the first time As to who at the DES is responsible for the changes on GMAG, several sources indicate that Shirley Williams, the Education Secretary, is taking a direct and personal interest in the new appointments. However, her advisers on GMAG remain in the shadows and do not appear to include GMAG's existing members

All this might be unimportant if it were not symptomatic of how GMAG handles its external relations. And external relations are important to a body whose decisions can affect the competitiveness of British biotechnology industry overseas. In this context, it is obviously desirable to get GMAG's standards accepted and applied by other countries. Unfortunately GMAG does not appear to communicate effectively with foreign scientific organisations. There is a strong feeling among some European scientists that GMAG could provide greater leadership in Europe by revealing more details of its work. As Dr Ravetz argues: "in about a year an American GMAG could be established that would operate in public and be easily accessible to foreign scientists. If that happens, the British GMAG would probably be ignored rather than be viewed as a model for all the world".

A. J. McClelland

Difficulties at PETRA worry designers of Europe's next accelerator

PETRA, the world's biggest storage ring for colliding electrons with positrons, is not behaving quite as expected, writes **Konrad Guettler**

THE European Committee for Future Accelerators (ECFA) convened its technical panel on the design of LEP—Europe's proposal for a 70 to 100 GeV electron and positron storage ring—in Rome recently, only to hear that the machine of which much LEP design has been based (West Germany's 19 GeV PETRA) is encountering difficulties.

Although PETRA started up very smoothly, ahead of schedule, and soon achieved beams of long lifetime, its luminosity (which determines the rate at which experiments can be done) is at present a factor of 100 or so below design The profound worry is that the scaling up of parameters from lower energy machines, such as the 10 GeV DORIS and SPEAR, to the very much higher energies of PETRA or LEP may in fact not be straightforward, or indeed possible at all It is still early days for PETRA but a large investigation program on both the technical and the theoretical side has now been launched both at DESY and by ECFA.

Nicola Cabbibo, a particle theorist at Rome University, and CERN are directing the attempts at increasing theoretical understanding of the observed beam properties. The main effects are the following:

- the maximum beam-beam tune shift 'ΔQ' is much less than its design value. ΔQ is the major factor, apart from the stored particle current, determining the machine luminosity, ie the number of interactions that take place at an intersection in unit time. The tune shift is a measure of the non-linear transverse forces between colliding bunches. All the existing machines turned out to have the same limiting value; and this ΔQ had also been assumed for PETRA and for LEP.
- Fast beam instabilities occur at various stored currents and appear to depend on the accelerating radiofrequency voltage. The circulating beams induce currents in the vacuum chamber walls and these wall currents create fields which interact directly with the beam. They can alter the normal betatron and synchrotron frequencies of the beam and thus cause instabilities.
- The accelerated bunches are larger than expected. (This affects the long-term beam stability.) Bunch lengthening is again due to the short-range fields induced by the bunch in the wall It leads to a wider energy spread within the bunch and can lead to head-tail instabilities.

There was a lot of concern at Rome that there was inadequate knowledge of beam dynamics at high energies. But a lot of experience is accumulating about high energy electron-positron machines and there was a widespread hope that while machine physicists have encountered very tricky problems indeed, they are unlikely to lead to profound revisions in the approach to LEP.

Since ECFA's Rome meeting, PETRA has run continuously and machine physicists have now pushed the beam current to a maximum of 18mA per bunch, almost up to the 20mA limitation design value. The previous current limitations have been overcome by changing the injection optics to the type also proposed for LEP The present aim is for fast injection and a high beam intensity, and only later will PETRA go for higher energy, and hopefully, higher luminosity. The latest machine runs at a centre-of-mass energy of 16 GeV have yielded 1-2 hadronic events per nanobarn cross-section per day—which can be compared with 20-100 events at DORIS. This is not a very high luminosity; but the DESY machine physicists are confident about increasing it Higher energies have to wait till next year when additional accelerating cavities will be switched in. □

Keeping down the cost of LEP

HIGH-ENERGY physicists have become very aware of current financial constraints, and are paying a great deal of attention to reducing the cost of LEP, without diminishing its physics potential. CERN has estimated the construction cost of a 70 GeV LEP to be a little over 1,000 MSF, which is almost the same as the SPS proton accelerator built at CERN a few years ago. No decision about the project has been taken, but ECFA hopes to present a detailed design study to the CERN Council by the end of 1979.

The physics interest in LEP is focussed on the maximum energy of the machine Lepton physics at high energies centres around the role of the intermediate vector bosons, the charged W^+ or W^- and the neutral Z°

Their masses can be estimated from the 'Weinberg angle' measured in neutrino interactions. At present predictions of the Z° and the W^+ mass are 89 ± 5 GeV and 78 ± 6 GeV, respectively. Single beams of about 45 GeV should be sufficient to produce Z°s in association with other particles but the full machine energy will be required to create a W^+W^- pair.

For technical and cost reasons, the original CERN design study (LEP-70, completed this summer) was optimised for an energy of 70 GeV; but it is now being redone for 80-85 GeV per beam. The size and cost of any such machine increase with its maximum energy, and LEP-70 already had a circumference of about 30 km. However, valuable experience was gained from the LEP-70 design exercise. In particular the cost sensitivity of machine parameters and components can now be assessed more reliably.

Wolfgang Schnell, of the CERN ISR division, has already proposed some interesting ways of reducing the construction cost of the future accelerator. LEP will have more than 2,500 dipole bending magnets to keep the particles on their orbits. The bending power of each magnet is low because too high a track curvature would make the electrons lose too much of their energy by synchrotron radiation. Conventionally, magnets are made by precisely stacking hundreds of thin steel plates and welding them together. But the low magnetic fields required for LEP (to reduce bending, synchroton radiation, and running costs) can be obtained by bonding a smaller number of steel plates in concrete, thereby dispensing with a lot of steel and fabrication cost.

Further, the particle beams have to be refocused repeatedly to counteract the natural tendency of beams to diverge. More than 800 quadrupole magnets are foreseen for this task. A similar number of higher multipole magnets completes the set of tools which allow machine operators to tune the beams and to overcome instabilities. Using anodized aluminium in the manufacture of quadrupole coils, in place of copper, would save much cost and production effort; detailed investigations into the properties of such coils are already under way.

The costliest item of the machine is the radio-frequency system which will accelerate the electron beams. Its components, klystrons and accelerating cavities, stretch over 1.5 km and their total power consumption at 70 GeV will be over 70 MW. About 19 MW of this will simply be lost in synchrotron radiation, a feature which is negligible in all existing proton accelerators. When a bunch of electrons passes through the cavities, it picks up energy from the applied RF power and thus is accelerated. But the four bunches in each beam spend most of their time outside the accelerating cavities, and the applied power is lost. An ingeneous method to reduce these losses is to couple a low-loss mode storage cavity to a set of accelerating cavities. Energy can then be saved by constantly transferring power between the accelerating and storage cavities.

By itself, this scheme would not help on component cost but it would either reduce running cost, or alternatively allow a higher maximum beam energy. superconducting RF cavities is cur-to become available in a few years' time; they will eventually allow the beam energy to be raised to about 130 GeV. The upper limit is set by the synchrotron radiation power that can be absorbed by the vacuum chamber, and by the field gradient ilmitations in the accelerating cavities. Research into super conducting RF cavities is currently carried out under a joint CERN-DESY programme, and a first test cavity is to be installed into DORIS by the end of 1979. However, it is unlikely that this technology will be sufficiently mastered for mass production for the first phase of LEP.

Great importance is also attached to the planning of experimental installations and facilities. Experience at DORIS and SPEAR has shown that the cost of experimental detectors and associated electronics and computers amounts to a sizeable fraction of the total machine cost. Furthermore, the average number of charged particles produced in an electron-positron annihilation at these energies is of the order of 20. Very complex detectors are required to track that many particles, and to identify them unambiguously. So a major effort must be made to reduce the cost of such devices.

Konrad Guettler

Fermilab looks to the future

David Dickson visits the Fermi National Laboratory and talks to its new director, Dr Leon Lederman (right)

THE past year has not been an easy one for the Fermi National Laboratory)Fermilab) at Batavia, just outside Chicago. Twelve months ago Fermilab's then director, Dr Robert R. Wilson, was pushing hard for the rapid completion of the next phase in Fermilab's development. This is to be the construction of a second ring of superconducting magnets which, with their increased field, will allow energies of up to 1,000 GeV to be reached, twice existing energies.

Last November, frustrated by indications that the Department of Energy would not provide him with sufficient funds for the rapid construction of the Doubler, Dr Wilson threatened to resign if such funds were not included in Fermilab's budget for the fiscal year 1979. But the department stood firm, providing Wilson with only $15 million of the $30 million that he had requested. And in February he resigned as threatened, citing the department's "indecisive and "subminimal" support for the Doubler project.

The whole affair left the US high energy physics community feeling somewhat uncomfortable. Many of those who had admired Dr Wilson's achievements in establishing Fermilab were uncertain about the strategy that he had chosen to adopt. Dr Wilson also left a legacy at the laboratory where many felt experimental programmes had been unfairly squeezed to allow development work on the Doubler to proceed as rapidly as possible.

The task of putting all the pieces back together again now rests with Fermilab's new director, Dr Leon Lederman, whose appointment was announced in October and who takes over the position full-time next 1 June (although already working several days a week at the laboratory).

Dr Lederman is currently professor of physics at Columbia University and director of the university's Nevis accelerator. He has also been closely involved with Fermilab since its inception; he was a member of the team which selected the site from over a hundred possibilities in the 1960s, and he also led the investigators who discovered the upsilon particle in the summer of 1977.

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Widely known not only as a successful experimentalist but also the owner of a quick wit—asked where the laboratory is going, his immediate response is that "It's going to stay in Illinois—I hope"—Dr Lederman has few illusions about the problems that lie ahead.

At the heart of many of these lies the issue of funding. Tight budgets have squeezed high energy physics as hard as other fields of science. Dr Lederman says that "basic research is being treated rather well by this administration"; but he points out that with three large accelerator projects now under way, the whole US high energy physics programme is "pushing very hard against its ceiling", and that "if the budget was even 5% higher, it would be an enormous relief".

High risks

The Energy Doubler will require a ring of 1,000 superconducting magnets, each 7 metres long. Although recognising that, with so little known about such technology, the field was one of high risks, Dr Wilson felt that the design of the magnets could be refined as the first ones were being produced (rather than waiting for a complete design before setting up production facilities).

However the gamble did not quite come off. Technical problems proved to be slightly greater than initially predicated—such as a slight movement in some of the wires of the coils, affecting their superconducting properties—and took longer than anticipated to perfect.

As Dr Lederman puts it: "There were some concepts in economy that were not realised. We had thought that a much larger fraction of the original magnets would be adequate and that we would be able to instal those to make up one sixth of the ring. But not enough of them were of good quality; and that created a problem with funding".

Dr Lederman places stress on the need to maintain a high level of performance, not only with the Doubler but also with the existing accelerator. "We have at present a responsibility to provide a large fraction of the US high energy research community with 400 GeV protons. I have found attention to the programme has slipped somewhat and people have been travelling here from the East coast and the West coast and waiting for a machine that was broken down a bit too often."

With development work on the Doubler's magnets now completed, a team at Fermilab is now preparing a final detailed design of the accelerator, which is expected to be ready in January.

Until a few years ago, many of Fermilab's problems might have been solved by a quick visit to sympathetic Congressman, with a few million dollars subsequently added to budget appropriations. But this style of approach is no longer appropriate, particularly as the decision making role has shifted from the old Congressional Joint Atomic Energy Committee—which produced much of the political support for early accelerators—to the Department of Energy's High Energy Physics Advisory Panel (HEPAP).

Earlier this year Dr John Deutch, director of the department's office of Energy Research, announced that the Office of Management and Budget had accepted a proposal to provide high energy physics with a constant budget over the next few years. Within this limit, the department has left it largely up to HEPAP to advice on how the resources should be allocated; the result is that any major development requires the active backing of HEPAP if it is to get support in the administration.

"One of our current very high-level efforts is therefore to bring the physics community into a level of acceptance, understanding and support of the Doubler project", says Dr Philip Livdahl, head of the project and currently acting-director of Fermilab.

Dr Lederman has already asked for an outside, independent review of the whole Doubler project, and has invited scientists both at Fermilab and in the universities to present ideas for the type of experiments that they would like to carry out when the 1,000 GeV level become available with the completion of the new ring.

A number of such ideas were discussed at a meeting of HEPAP which was held at Fermilab at the beginning of last week. It is said that the exercise had a beneficial effect on some of those who still have doubts about the Doubler project. The advisory panel has now asked the laboratory to prepare a detailed report on the ways in which the Doubler construction programme might be integrated with other activities, setting out the option at different budget levels so that it can assess the requests for greater support.

Colliding with Japan

From Fermilab's perspective, HEPAP's support is likely to be particularly important next year, since it is hoped that funds to begin construction of a colliding beam facility—involving collisions between particles in the existing ring and those circulating in the superconducting ring—can be included in the Department of Energy's budget request for 1981.

One bright spot on the funding horizon is the proposed collaboration with Japanese scientists. High energy physics figures prominently in a programme of research collaboration in the energy sciences between the US and Japan that could be worth $ 1,000 million over the next ten years.

Collaboration in high energy physics will take place at three different levels: carrying out joint experiments on existing facilities, joint research and development in accelerator design and technology, and—potentially the most ambitious point—funding of new facilities.

In the first category, Dr Lederman hopes the Japanese contribution will add those few percentage points to the research budget which would have a magnified effect on scientific productivity. As to the third, Japanese funds could help bring forward some of Fermilab's more long-term plans: in particular, the development of facilities for the production of high-energy anti-protons which could be injected into the same ring as the protons, but circulating in the opposite direction to give collision energies of 2,000 GeV.

World Machine

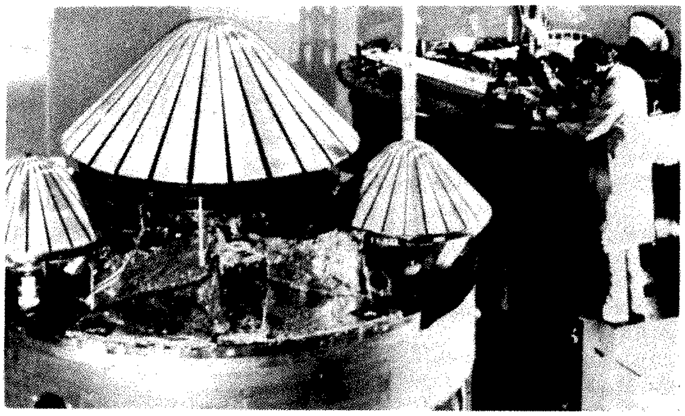
And there is an interest in high energy physics not only in Japan, but also in the Republic of China, which has recently sent a number of scientists to study at Fermilab. Dr Lederman has been closely involved in the activities of the International Committee for Future Accelerators, and in particular its scheme for the possible construction of a "world accelerator" in the 20,000 GeV range (20 TeV), well beyond the economic scope of any one country.

"When we started there were only three areas of the world interested in the possibility of such a project: the US, the USSR and Western Europe. With three there are problem of cooperation; but with five the situation is much better, since it does not matter too much if one decides to drop out"

Dr Lederman is also understandably enthusiastic about the particular possibilities raised by the Japanese connection, and is a member of the Department of Energy working party which has been discussing details of the proposed collaboration between the two countries with Japanese scientists.

He feels that high energy physics could play an important role in Japanese society. "Talking to Japanese politicians and ministers, they seem to feel the lack of intellectual heroes, the role played by Einstein, Fermi, Newton and so on in the West. They do not have that heritage to inspire the young people." High energy physics, Lederman belives, might provide the inspiration. □

news in brief



Before the launch: checking out the probes that have now surveyed Venus's atmosphere.

Exploration of Venus: Ten spacecraft—six American and four Russian—are exploring Venus this month. One spacecraft, the US Pioneer Venus I, went into orbit on 4 December and is expected to be operational for at least a year. Five other US probes for exploring the Venusian atmosphere penetrated the atmosphere on 9 December and transmitted data during their hour-long descent to the surface. They were not expected to survive the crash landing, though one continued to radio information back to Earth for an hour after reaching the surface. The probes contain instruments to measure density, pressure, wind speeds, heat flow and atmospheric composition.

Meanwhile, towards the end of December, two Soviet spacecraft will "fly-by" Venus, and each is expected to release a lander. These landers are intended to survive for a short period on the surface of the planet and take photographs of their landing site. The instruments on the Soviet probes will need to withstand the extreme conditions on Venus: atmospheric pressures 100 times that on Earth, high concentrations of sulphuric acid in the air and temperatures of nearly 500 °C.

"Anti-intellectualism" hits US basic research: Federally-supported basic research in the US is feeling the edge of a definite anti-intellectualism in some sectors of American public opinion, according to Dr Richard Atkinson, director of the National Science Foundation, in a speech at the University of Indiana. He added, however, that, in supporting those who attacked esoteric-sounding research programmes, the public seemed to be demanding some assurance that Government-supported activities were connected with real human concerns and problems. "The scientific community will be far more likely to gain acceptance by dealing with these concerns in a patient and serious way than by reacting in an ill-tempered or patronising fashion."

Dr Atkinson, whose agency received a budget cut in Congressional committees that reduced the growth of its basic research to below the level of inflation, said that the importance of basic science to the economy was now being recognised in China after the Gang of Four's hostility to scientists and scholars. "The Chinese experience should be a sobering reminder to all of us, scientists and non-scientists alike, that intellectual creativity is every bit as significant as a measure of a society as military strength or economic output," he said. "It seems somewhat paradoxical to me that the present Chinese leadership has grasped this lesson so thoroughly at a time when American opinion seems increasingly uncertain of the value of basic research and scholarship."

European control of genetic manipulation: Guido Brunner, EEC Commissioner for science and technology, has suggested that the Community should formulate rules to cover the experimental and industrial use of genetic manipulation. All such work would have to be notified to the national authorities of the member countries, he said. At the moment, Britain is the only one which requires notification, but others are planning to introduce legislation. A spokesman said the Commission was only at the stage of talking about the proposals, and it is not known if it intends to set up a committee similar to Britain's Genetic Manipulation Advisory Group.

Government calls for cut-backs in animal experiments: Scientists in Britain are being urged by the government to restrict the use of living animals in experiments. In a letter to all scientists in the country licensed to carry out experiments on living animals, the government says the experiments should be "limited to the minimum compatible with the pursuit of legitimate scientific ends". The letter stresses the importance of finding alternative methods of conducting scientific experiments, such as tissue culture or computer modelling. Scientists are asked to take "every reasonable step to confirm, before using living animals, that their investigations cannot be done by any alternative means".

The government hopes scientists will be encouraged to develop new alternative techniques. But, Dr C. Stratmann, scientific consultant to the Fund for Replacement of Animals in Medical Experiments (FRAME), said: "We are concerned that little funds are available specifically to find alternatives." Over the last ten years, FRAME has built up a library of information and literature on alternatives. Dr Stratmann urged scientists to contact FRAME if they wish to find out about alternatives.

The number of experiments on animals has remained fairly constant over the past eight years—at about 5.5 million experiments a year, according to government statistics on animal experiments published last Thursday. Over half of the experiments were carried out for commercial reasons such as drug and cosmetic testing.

Inquiry into extension of Natural History Museum: The government has called for a public inquiry into the Natural History Museum's plans to build a new wing known as the East Infill Block. The plan has aroused public interest because it involves demolishing part of the original Waterhouse building, which is listed as of historical interest. Although an inquiry is not required by law, the government wants everyone concerned to have a chance to put their case.

Pesticides in the diet : The average British diet still contains unacceptably high levels of Dieldrin, a pesticide used against woodworm, according to the latest report of the Government Chemist. Although the level of Dieldrin in the diet is less than half the Acceptable Daily Intake recommended by the World Health Organisation, it has not declined in recent years, despite increased restrictions on its agricultural use. Dieldrin is one of the most poisonous organochlorine pesticides, especially as it is not metabolised by the body.

The persistence of Dieldrin in the diet suggests that it may come from a non-agricultural origin. In an isolated incident earlier this year, 4,000 chickens died in East Anglia after eating litter containing wood chips treated with Dieldrin. Government chemists, therefore, began an inquiry into whether some cattle feeds made from dried poultry litter could be a source of Dieldrin in milk.

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How molecular biology won its place in the USSR

PROFESSOR Lev Tumerman, who played a leading role in the fight against Lysenkoism, emigrated from the Soviet Union to Israel in 1972. Since then, he has been prominent in the press discussion of the Kyshtym nuclear "disaster" of the 1950s. Vera Rich talked to him recently at the Weizmann Institute, where he now teaches.

PROFESSOR Tumerman was originally a physicist. He later turned his attention to molecular biology—a change which, he explained, reflected the whole story of his life in Russia.

Owing to the stress placed by Soviet defence policy of the early 1950s on the atomic bomb, physicists enjoyed what was, for those days, a relatively privileged position. They had greater freedom of action than other scientists and according to Tumerman, played a leading role in the fight against Lysenko.

In the early 1950s a number of physicists became interested in molecular biology and started working underground. Frequently, said Tumerman, the physicists acted as hosts to such unauthorised gatherings. "Timofeev-Resovskii's first lecture on genetics was given in Kapitsa's Institute of Physical Problems", he said. "I myself organised the first seminar on genetics at the Lebedev Physical Institute. We had about 200-300 participants at every meeting of the seminar. This was the first legal seminar on genetics. The time was ripe to begin the fight".

Many famous physicists, including Kurchatov and Tamm, were by now interested in molecular biology, and the pressure was kept up in many forms. An underground seminar was held every year in the Ilmensk nature reserve.

By this time, Professor Tumerman's interests had switched almost completely to molecular biology, although this change was not reflected in his official work. "Before my arrest", he explained casually, referring to his seven years in solitary confinement, "I had worked for 20 years with Sergei Vavilov, as a pure physicist. (Sergei was the brother of Nikolai Vavilov, you remember, who perished during the Lysenko period). We worked together from 1926 to 1947 on fluorescence as a physical phenomenon. The first fluorescent lamps in Russia were made by my hands."

Genetics—learnt in gaol

"In the Vladimir prison, there was a very good library—built up from books confiscated from the homes of prisoners. I began to read books on genetics. There was one very good book by Sunnot and Dent. These books impressed me so much that after I was released I changed my field of interest. I felt that purely physical phenomena were no longer of interest to me. I could only work at the interface of physics and molecular biology. I wanted to understand the role of physics in biological processes. So from 1954 to 1958, I worked at the Lebedev Physical Institute, but I changed my field of activity to photosynthesis. This was my official work, but unofficially I was interested in the whole problem of genetics and molecular biology."

In the seminars which Tumerman and his fellows organised, little could be done except to present and review the results of foreign papers. Yet, although no experimental work could be done at that time in molecular biology, Tumerman sees the seminars as far from fruitless. "We felt it was very important to involve young people in this field—to give an impetus to press the authorities. And in 1958 we won our victory, and molecular biology was allowed to exist officially."

As an immediate consequence of the new policy, Academician Engelhardt was given the task of setting up a new Institute of Molecular Biology, and Tumerman himself was invited to be head of the Laboratory of Bioenergetics. This laboratory investigated a number of problems, including nucleic acids and photosynthesis; Tumerman's own work was an attempt to apply physical mechanisms to problems of molecular biology.

"During the last few years", Tumerman added, "genetic engineering has evolved. But this came up after I had emigrated and I know nothing about it. I never worked on military applications. It was not easy for me—they often pressed me, but I managed to stay clear of military problems."

Yet although his own work remained unclassified, Tumerman did in fact come into possession of certain data which, whether technically classified or not, the Soviet authorities clearly wanted to keep secret. This was the story of the Kyshtym disaster.

"In the early 1960s", explained Tumerman, "Timofeev-Resovskii was working at the Inmensk nature reserve on the action of radiation on plants, and there was certainly no contamination there". (This is the nature reserve where the clandestine meetings on molecular biology and genetics had been held.)

At that time, Tumerman's brother was working as a construction engineer on the new atomic power station at Beloyarsk, some 100 km away. One day, Tumerman set out to visit his brother by car, leaving at midnight. "By 5.30 it was light", he recalled, "and I saw a sign by the road 'No stopping for 25–30 km'.

"On both sides there was *mertvaya zemlya* (dead land). No villages, only chimneys—the houses had been pulled down to keep the people from returning".

Asked to clarify the expression *mertvaya zemlya*, Tumerman explained that by 'dead land' he did not mean there was any signs of devastation. The land was simply empty—no agriculture or any other sign of land-use. "The local people said it was due to a 'catastrophe' at Kyshtym", he said. "I don't have any knowledge of its cause or nature. It had been some years before. I assumed that some waste must have fallen and that the level was so high that the people had had to be evacuated and many hundreds of square kilometres of land left free from cultivation".

Keeping quiet about Kyshtym

Tumerman at first made no attempt to publicise this knowledge. Only when, some years after Tumerman settled in Israel, Zhores Medvedev began to draw Western public attention to the disaster, did Tumerman begin cautiously to publish what he knew.

"I was afraid," he explained, "that people would use the story to interfere with the building of atomic power stations. I believe that the building of atomic power stations—real power stations, not plutonium processing plants—is the prime necessity for mankind. We must be prepared that in maybe 20 years the oil will run out. And world politics will change. No one will be interested in the Middle East".

"I published my account", he insisted, "to prove that the danger is not in atomic power stations. Atomic power stations are the safest form of energy production, much safer than oil".

However one accepts this estimate, Professor Tumerman's assertion that Kyshtym was not due to some power station disaster seems, on the face of it, irrefutable. For at the time when he passed through the devastated area, he was on his way to visit his brother at Beloyarsk, where the first atomic power station in the Soviet Union was "only at the first stage of building". "So whatever caused the disaster", Tumerman concluded, "it simply could not have been connected with any power station!" ☐

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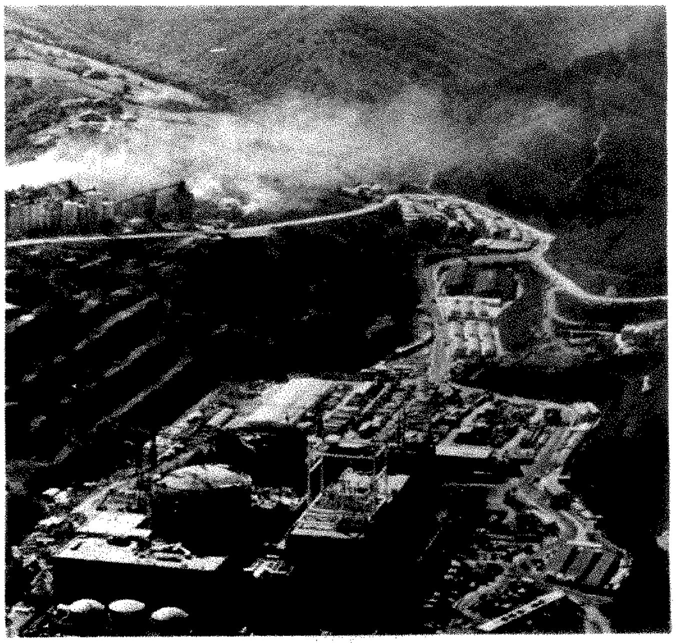

On cave paintings and nuclear security

By Alvin Weinberg, director of the US Institute for Energy Analysis, Oak Ridge, Tennessee

Dr J. Altuna took well-justified pride as he showed us the cave paintings in the Ekain caves near San Sebastian that he and his colleagues had discovered in 1968. The horses (pictured above) by some Cro-Magnon genius 11,000 years ago were in every respect remarkable: according to Dr Altuna, the best cave paintings of horses to have been discovered to date. The paint we now see is largely manganese dioxide which was probably mixed with animal grease to apply it to the wall.

I was in the Basque country at the invitation of Aranzadi, the Basque Scientific Society. I was to participate in a discussion of nuclear energy with Professor Erik Arrhenius of Sweden. My reaction to the cave paintings, aside from astonishment at the skill of the artist, was therefore coloured by the nuclear debate. Could these artefacts of man, some of which have lasted as long as 11,000 years with little deterioration, bear on the disposal of nuclear wastes? Dr Altuna was very doubtful—after all, not all the paintings are in perfect condition. On the other hand, some were; and in the absence of proof of the unprovable—we cannot know *absolutely* the fate of geologically sequestered wastes 10,000 years from now—we can draw inferences from long-lasting artefacts of man. Herbert Muller, in his *Freedom in the Ancient World*, speaks of the ancient cave-artists as having "unconsciously . . . worked for posterity". In this instance they have, unwittingly, proved that at least some works of man can survive for immensely long times; 11,000 years is sufficient for the radioactivity in wastes to fall below that of the original uranium ore whose fission gave rise to the wastes. And if *some* artefacts can last that long without change, is it not more plausible to believe that we, who are more sophisticated than our Cro-Magnon ancestors, can reproduce the necessary conditions for survival of solidified wastes than that we cannot?

Ekain, or the 30,000-year-old stone Venuses, or, for that matter, the 2,000-million-year-old natural reactors at Oklo in Gabon add to what I believe is already convincing evidence that waste disposal is tractable. The issue ought not to be used as an argument against the use of nuclear power, despite the controversy that once again rages over the feasibility of waste disposal.

Rather, the central argument, and the one that I suspect all the other issues will coalesce around, is the peculiar requirement for institutional stability demanded by nuclear power. It is therefore ironic that just 30 kilometres from Ekain, with its evidence for the technical feasibility of waste disposal, lies Lemoniz, a prime reminder that nuclear energy and social instability don't mix. About a year ago, a main steam generator in one of the pressurised water reactors under construction at Lemoniz was bombed by Basque terrorists; and a terrorist was killed in a shoot-out with guards at the construction site.

The Lemoniz incident was not primarily anti-nuclear: it was rather that Lemoniz (above right) was sited under the Franco regime without much consultation with the local Basques. It had become a symbol of Basque nationalism: in the view of many Basques, Madrid was imposing its will on them.

This essentially political objection to Lemoniz provides fertile ground for anti-nuclear activism. And in the colloquium, Professor Arrhenius and I were bombarded with the same questions I encounter everywhere: waste disposal, reactor safety, proliferation, low-level radiation effects.

The Basques are of two minds about Lemoniz. On the one hand, they see it as imperialist intrusion. On the other, they sense that nuclear stations, rather more than, say, oil-fired stations, confer a degree of energy autarky. Once the Lemoniz reactors are built, the Basque country will have a large source of electricity entirely within Basque borders. A precondition for this additional degree of energy self-sufficiency, I pointed out to the Basques, is an end to nuclear terrorism. Once Lemoniz is operating and its core contains billions of curies of radioactivity, it must be judged out of bounds for would-be terrorists: a core meltdown induced by terrorist action would harm the Basque country more than anywhere else.

Would the presence of an operating reactor mitigate the scope of action of terrorist groups—at least to the extent of putting nuclear plants out of bounds? I should think this might be the outcome in the Basque country where terrorists and local population presumably have the same aim—more autonomy for the Basques. Once Lemoniz is operating, one would expect terrorist acts against it to cease. I am less sanguine about nuclear terrorism in other situations where the terrorists may have no motive except destruction of the existing order. The only safeguard against such terror is heavy security. It is no accident that Lemoniz is as closely guarded as any US atomic weapon establishment. But these security measures are confined to the reactor site, and to speak of such measures as implying a police state seems to me to be an absurd exaggeration. Heavy security against terrorists is a price that nuclear energy exacts; fortunately, the number of places that will have to be so guarded is small, and they pose little threat to the society as a whole. I would hope that nuclear plants everywhere are made as secure as Lemoniz now is. ☐

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correspondence

Good and bad Nobel prizes

SIR,—The Soviet authorities and mass-media are distinguishing between 'good' and 'bad' Nobel prizes. A Nobel prize is a good one and means great and deserved recognition for the USSR, if it is awarded to an obedient and loyal Soviet intellectual: to the writer Sjolokhov, to the physicists Basov and Prokhorov, to the chemist Semjonov or the economist Kantorovich.

The Nobel prize is a bad one and is a provocation of western imperialists and international reaction, if it is awarded to some of the Soviet writers or scientists, who are on the 'banned' index; say Boris Pasternak, Alexander Solzhenitsyn or Andreii Sakharov. Pjotr Kapitsa's recent Nobel prize for physics is considered good by Soviet authorities and mass-media.

Professor Kapitsa is no doubt a great and famous scientist. He was awarded the Nobel prize for a discovery he made 40 years ago in Moscow with equipment which had been sent to him by his professor, Ernest Rutherford, from Cambridge. But is this year's Nobel prize really a good one for the Soviet Union? Does Pjotr Kapitsa really belong to the obedient and loyal Soviet intellectuals?

The facts of his life suggest the opposite. The young Soviet physicist came to Cambridge in 1921 and in a few years became Rutherford's most respected collaborator and expected successor in the Cavendish Laboratory. In 1934 Kapitsa left for a vacation in the Soviet Union and unexpectedly was prevented by Soviet government from returning to Cambridge. The Soviet government offered him a new institute in Moscow without any financial and administrative restrictions.

Rutherford had been trying very hard to get his collaborator back, but when he finally saw the vanity of his attempts, he finally decided to make a beautiful gesture: he sent the equipment of Kapitsa's recently-built Mond laboratory to Moscow. Kapitsa had just completed the equipment before his departure on vacation.

Kapitsa had his institute in Moscow, an important position in the Soviet academy of sciences, and a considerable influence in the government when, in 1939, another of the future Nobel laureates and his close friend Lev Landau had been arrested. Kapitsa came to the Kremlin and presented an ultimatum: if Landau was not released, Kapitsa would leave his institute and stop his work. This was a risky step, but the Soviet government needed Kapitsa. Landau was released and his life saved.

In spite of all this Kapitsa was himself a privileged prisoner. Until the late fifties he was not allowed to travel abroad, his contacts with foreign scientists were restricted, and his correspondence often confiscated. Kapitsa became a prisoner in his country house at Nikolina Gora in 1945, when he fell out with Stalin over the problem of Soviet nuclear weapons. He was released from prison only after Stalin's death and only then was able to resume his directorship in the Institute of Physical Problems.

Kapitsa never hid his critical attitude to the Soviet reality, nor his sympathy with those who openly oppose this reality. "How many roubles would our finance ministry release to Rembrandt for the brushes, how much for the canvas and how much for the colours?" says Kapitsa, chiding administrative restrictions confining Soviet science. "On 20 February 1956 an instruction was issued, according to which all directors of academic institutes must present a plan of every single working day for the approval of the academy. But I, for example, cannot plan every single working day, say when I will be sitting in the laboratory and when in my studio—this depends on how our investigations will proceed . . ."

Very sharply, and often, Kapitsa criticised the backwardness of Soviet science, its low productivity. In 1965 the USSR and USA had approximately the same number of scientists, but the USA did about one-third of world science, and USSR only one-sixth. "Therefore, no matter how sad it may sound, we have to admit that the productivity of our scientists is two times lower than that of Americans."

Andreii Sakharov is a good friend of Kapitsa, and so are many other Soviet dissidents. Kapitsa was one of the initiators of the public protest which helped to release Soviet biologist Zhores Medvedev from psychiatric prison. His signature was among the few tens demanding the abolition of the death penalty in the USSR. And his villa in the institute park is a frequent meeting place for dissident Soviet intellectuals. The atmosphere inside is stimulating: in Kapitsa's studio hangs a photograph of Solzhenicyn, with his handwritten dedication.

When the Soviet sculptor Ernst Neizvestnyj was expelled from the USSR in 1974, Kapitsa gave a farewell party for him in his house. In the presence of many guests he made a toast, in which he said: "I have to admit that I envy you a little bit. You are young and you will live to the time when you could come back to Moscow. And you will walk on the Solzhenitsyn square, and on Andreii Sakharov prospect, and the street where you will have your atelier will bear your name. But I am already old and will hardly live to see it . . ."

Eighty-four years' old academician Kapitsa may really not live long enough to see such changes in USSR. But when they finally come, nobody will deny his merits.

This year's Nobel prize for Pjotr Kapitsa is, therefore, not quite a 'good' prize for the Soviet Union. But is more than a highly deserved prize for an old professor.

Name and address withheld by request

Heraclitus Revisited

SIR,—You may be interested in these lines stimulated by the review of Erwin Chargaff's new book (*Nature* **276**, 133; 1978):

I'll tell thee everything I can:
  There's little to relate.
I met an old, embitter'd man
  Bemoaning his sad fate.
"Who are you, aged man?" I said,
  "And what is your pet peeve?"
His answer trickled through my head
  Like water through a sieve.

He said "I met a fervid pair
  Who built a model crude
The younger one had lengthy hair
  And both of them were rude.
They claimed that they were out to find
  What makes genetics tick
And when I call the scene to mind
  The mem'ry makes me sick."

But I was thinking of a plan
  To write a grant request
And if the funding once began
  I'd count myself well-blest.
So, having no reply to give
  To what the old man said
I cried "Come tell me why you grieve"
  And thumped him on the head.

He said "The times are bestial
  The world has gone to hell.
My culture is celestial
  In science I excel.
Of Mozart's symphonies and themes
  I know the form and keys.
I dream the most exalted dreams
  Of any Viennese."

But I was thinking of a way
  To cut him down to size
By noting what he had to say
  Was something less than wise.
And then I wondered if these lines
  Would capture his attention
Because his musings show some signs
  Of scanty comprehension.

And so if e'er by chance I put
  My fingers into glue
Or madly squeeze a right-hand foot
  Into a left-hand shoe
Or if I drop upon my toe
  A very heavy weight
I weep, for it reminds me so
Of that old man I used to know
Who seems distracted with his woe,
Who mumbles of the long-ago
As if his mouth were full of dough
  Bemoaning his sad fate.

The Wight Night

THOMAS H. JUKES

University of California,
Berkeley, USA

Homo erectus

SIR,—I was very pleased to see that due recognition was given to *Homo eructus* in the item on East Javan hominids (28 September, page 306).

Few, indeed no, previous commentators have given this hominoidal form sufficient weight, although his more commonly used name gives a clue to his place in taxonomy: just a breath away from Pe-King Man must come Bel-Ching Man.

His undoubted place as a direct ancestor of *H. sapiens sapiens* is attested by *H. eructus'* easily identified descendants in any group of humans today.

However, I have been greatly puzzled over how his patronymic attribute has been deduced from skeletal evidence.

N. R. LANDON

London, UK

news and views

Solar energy storage and conversion by hydrogen cycle

from Robert W. Cahn

All forms of 'current' energy—Sun, winds, waves, tides—are intermittent and variable and their use for large-scale and domestic purposes alike demands provision of energy storage. This may well turn out to be more difficult than and just as capital-intensive as the devices for absorbing and converting the energy in the first place. The recent controversy in *Nature* concerning the storage requirements associated with a system of windmills indicates the crucial importance of the storage capacity if steady power is to be generated from variable input.

Most domestic solar heating installations to date are intended largely or wholly for water-heating, and the thermal mass of an insulated hot-water tank provides all needful storage capacity. If, however, space-heating is proposed as well, or even electricity generation, then a more sophisticated storage facility is needed. Heat can be stored in pebble beds, water tanks or salt baths and retrieved by forced heat exchange when required. However, space is expensive and a high storage capacity per unit volume (specific capacity) is therefore at a premium. A high specific capacity implies exploitation of chemical reaction energies as distinct from simple specific heats, and it is in this connection that metal hydrides have received a good deal of attention in the past four years.

A number of metal hydrides (TiFe, $LaNi_5$, Mg_2Ni and Mg_2Cu are the most studied) have convenient dissociation pressures and temperatures, substantial heats of dissociation and can store more hydrogen per unit volume than can a liquid-hydrogen Dewar vessel (Hoffman *et al. Int. J. Hydrogen Energy* 1, 133; 1976). A number of laboratories, of which Brookhaven National Laboratory has been the most active, have been examining the metallurgical and engineering features of hydride storage installations, partly with automotive uses in mind. A recent paper from Argonne National Laboratory (Green, Schreiner & Shaft *Int. J. Hydrogen Energy*, 3, 303; 1978) presents a conceptual study of a system using such hydrides for storage of heat and its use in adjustable proportions for domestic heating, air-conditioning and electricity generation. It is a welcome publication, since much work in this field is issued in inaccessible reports or expensive conference proceedings — what might loosely be termed scientific *samizdat*. All 12 references at the end of this paper are to unpublished reports, house journals or conference proceedings, and this practice leaves little scope for the serendipitous browsing that so often generates new scientific ideas. For this reason, the existence of regular journals like the *International Journal of Hydrogen Energy* is particularly welcome; in a field like this, the absence of a highly specialised journal is apt to be associated with entrenched scientific *samizdat*.

The Argonne apparatus, in the simplest of its several forms, involves a solar collector with concentrator that can warm a heat-transfer fluid to 140 °C. The warm fluid then heats up a primary $LaNi_5H_6$ bed, releasing hydrogen at about 50 atm. The released hydrogen is cooled to 50 °C and expanded to a pressure of 40 atm or less. The cool, lower-pressure hydrogen is then converted to hydride in a secondary bed (a cheaper bed using mischmetall, a mixture of rare earth metals, instead of lanthanum, is proposed). The heat released during expansion and hydration is absorbed in a separate loop of heat-transfer fluid, and this heat (at 50 °C) can be used for space-heating. Hydrogen is released from the cooled $MmNi_5H_6$ secondary bed by lowering the pressure to about 8 atm and can be recombined with the primary $LaNi_5$ bed at 50 °C at the same pressure. That bed is then reheated by solar energy and the cycle recommences.

This represents a simple non-storage cycle. In fact, the storage bed would be kept hydrogenated till nightfall and the apparatus would then be operated in heat-pump mode; the primary bed is kept at 50 °C by manipulating heat-transfer fluid and is used as a source of space heat, while the secondary bed can progressively dehydrogenate at low pressure and 0 °C; this low temperature is achieved by absorbing heat from the environment.

To achieve continuity in operation (as would be required for continuous air-cooling or for electricity generation) at least two primary and two secondary beds would be used, with appropriate switching of hydrogen and heat-transfer fluid flows so that each undergoes a staggered cycle: absorption, heating, desorption, cooling. The cycle length is presumably limited by maximum heat-transfer rates at one extreme and by low demand for domestic heat at the other extreme. A variant of this cycle allows the apparatus to be used for air-conditioning instead of space-heating.

A more complex system involving three beds of each kind would permit a proportion of the absorbed solar energy to be used for electricity generation. (Warm hydrogen would be passed through a turbogenerator: the alternative of oxidising the hydrogen to water in a fuel-cell would be inappropriate in a closed cycle system). The authors undertake a detailed assessment of realistic efficiencies to be expected, allowing for heat losses involved in heating and cooling the storage beds: the maximum theoretical efficiency is 28% (electric power as a fraction of solar heat absorbed by the primary bed), the expected realistic efficiency is 16.5%. If the apparatus is used simply as an air-conditioning (cooling) device the heat-pump mode ensures that, at ambient temperature, for each mole of hydrogen about 3.3 times the dissociation energy can be extracted as useful cooling power.

Nothing is said about economics. The high cost of mischmetall and even higher cost of pure lanthanum would seem to place a question-mark against the particular hydrides chosen, in spite of their particularly convenient dissociation temperatures.

The Argonne design prompts a number of reflections. The apparatus would be of very considerable com-

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plexity Each bed would be associated with several heat-transfer loops, pumps and pressure valves, and there would need to be an elaborate automatic control installation to switch both hydrogen and heat-transfer fluid into appropriate circuits at appropriate times, to secure the staggered cycles and also in response to changes of incoming solar power level, space-heating or cooling demand, hydride temperature, hydrogen pressure and fluid temperature in various parts of the apparatus This is quite apart from the control provisions needed to keep electric output constant in the face of varying hydrogen temperatures and pressures. There is a widespread illusion that renewable energy sources such as Sun and wind are necessarily associated with primitive or intermediate levels of technology: people are apt to think in terms of solar cookers for use in Indian villages or of water-heaters for swimming-pools or for Israeli dwellings. In fact, if solar power is ever to be more than a negligible fringe energy source, plainly it must be accompanied by very advanced technology This is true both of the initial capital input (such as the manufacture of silicon solar cells) and of the maintenance of complex installations, which will place high demands on initial design (perhaps in the form of replaceable modules), on fault diagnosis and on high reliability in manufacture. Such things will certainly be 'mills' in William Blake's sense, though bright and beneficial rather than dark and satanic

In the very early days of domestic electrical power, a century ago, there was a brief sharp argument as to whether separate electric generators were to be installed in the basement of each home, or central generation and distribution was preferable One may wonder whether, before another century is out, this argument will be reopened in sunny parts of the world. Meanwhile, the most promising application, in the short term, of installations like the Argonne design would be for air-conditioning in hot, isolated and fuel-deficient places like North-western Australia. Such an air-conditioning facility, built into dwellings and communal spaces, may well be the factor that determines whether or not labour can be attracted to work in mines in such inhospitable places ☐

Sickle cell: a metastable disease

from Robin Briehl

SICKLE-cell anaemia was the first 'molecular disease' to be discovered; the term was coined originally for it by Linus Pauling to celebrate the discovery that the condition was linked to the presence of a mutant haemoglobin in the red cells of the patients In the quarter century that has passed efforts have been made, with less than conclusive results, to understand in detail just how the deviant solubility properties of the abnormal haemoglobin bring about the sickling phenomenon, and what, in the light of such a biochemical or biophysical understanding, one could hope to do about it. I want to consider here one aspect of this multifaceted problem

In the classical picture the pathogenesis of sickle cell disease depends on deoxygenation-dependent polymerisation of the abnormal haemoglobin S into long fibres and the formation from these of anisotropic, spindle-shaped, liquid crystalline bodies, designated tactoids. The final result is a viscous 'gel' which deforms the erythrocyte and makes it rigid. Gelation and its inhibition have been studied intensively in the past 5 years by attempts to develop covalent or non-covalent inhibitors of gelation, by elucidation of the structure (see *News and Views* **272**, 476; 1978) of the intermolecular interactions in the fibre, and, the subject of this note, by studies of the kinetics and equilibria of gel formation.

Under the currently accepted model for the kinetics of gelation, a long stage of highly cooperative nucleation is followed by rapid fibre growth and then by a slow alignment of fibres to the anisotropic state[1] The termination of the nucleation or latent stage, originally observed through an increase in viscosity[2] is also manifested in heat absorption, the onset of birefringence[1] turbidity increase[3] and broadening of water proton magnetic resonance linewidths[4]. The activation energy for nucleation is about $90\,\mathrm{kcal\,mol^{-1}}$. During the latent stage the supersaturated solution of haemoglobin monomers (that is, tetramers, molecular weight 64,500) is unstable with respect to the phase of isotropically oriented fibres. The latter, in turn, is unstable with respect to the formation of the anisotropic state, as evidenced by the slow development of birefringence

Minton[5] has applied fundamental models for the entropically driven formation of tactoids from long rod-like molecules or particles to the haemoglobin S system to develop an equilibrium model of gelation. The non-ideality which arises from the large excluded volumes of the highly asymmetric polymers results in a phase diagram containing regions of purely monomeric haemoglobin and of isotropically and anisotropically oriented fibres, the last either in the tactoidal state or in a very highly aligned and concentrated quasi-cystalline condition. The diagram also contains a stem-shaped heterogeneous region reflecting a separation into conjugate isotropic and anisotropic phases.

Pumphrey and Steinhardt[6] have now discovered and characterised a new state of haemoglobin S which defines a new metastability as well If concentrated solutions of deoxyhaemoglobin S are stirred above a low threshold rate, birefringent, uniaxial, needle-like crystals appear instead of the familiar gel Since seeding of a gel with crystals results in conversion to the crystalline state, the crystal form is more stable thermodynamically, although only marginally so, since the well-defined solubility of the crystals in very near the minimal gelling concentration. Our observations[7] on dilute gels of deoxyhaemoglobin S, observed in 1.7 M phosphate, show a similar metastability: As a function of rates of warming and shaking, there is reversible formation of either a gel or a fluid but more turbid suspension.

In addition, polymorphic forms of the fibre exist (see *News and Views, op. cit.*), so that structural as well as physical chemical studies demonstrate

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1 Hofrichter *et al Proc natn Acad Sci U S A* 71, 4864 (1974)
2 Malfa & Steinhardt *Biochem. biophys Res. Commun* **59**, 887 (1974)
3 Moffat & Gibson *Biochem biophys Res Commun* **61**, 237 (1974).
4 Eaton *et al Biochem biophys Res Commun* **69**, 538 (1976).
5 Minton *J molec Biol* **82**, 483 (1974)
6 Pumphrey & Steinhardt *Biochem biophys Res Commun* **69**, 99 (1976), *J molec Biol* **112**, 359 (1977)
7 *Proc Symp Molecular & Cellular Aspects of Sickle Cell Disease* 145 (1976)
8 Magdoff-Fairchild *et al Proc natn Acad Sci U S A* **73**, 990 (1976)
9 Hofrichter *et al Proc natn Acad Sci U S A* **73**, 3035 (1976)
10 Goldberg *et al J biol Chem* **252**, 3414 (1977)
11 Briehl *J molec Biol* **123**, 521 (1978)
12 Ross *et al J molec Biol* **96**, 239 (1975); **115**, 111 (1977).
13 Briehl & Ewert *J molec Biol* **80**, 445 (1973)
14 Shibata *et al J biol Chem* **252**, 7468 (1977)
15 Swerdlow *et al Hemoglobin* **1**, 527 (1977).
16 Harris & Bensusan *J Lab clin Med* **86**, 564 (1975)
17 Gill *et al Science* **201**, 362 (1978).
18 Minton *J molec Biol* **110**, 89 (1977)
19 Ross & Minton *J molec Biol* **112**, 437 (1977)
20 Williams *Proc natn Acad Sci U S A* **70**, 1506 (1973)
21 Bookchin *et al Nature* **256**, 667 (1975)
22 Waterman & Cottam *Biochem biophys Res Commun* **73**, 639 (1976)
23 Benesch *et al Biochem biophys Res Commun* **81**, 1307 (1978)
24 Behe & Englander *Biophys J* **23**, 129 (1978).
25 Kowalczykowski & Steinhardt *J molec Biol* **115**, 201 (1977)
26 Eaton *et al Blood* **47**, 621 (1976).
27 Sunshine *et al Nature* **275**, 238 (1978)
28 Hermans *J Colloid Sci* **17**, 638 (1962)
29 Allison *Biochem J* **65**, 212 (1957).

the existence of different condensed states.

The most common assay of gelation, minimal gelling concentration, depends in part on kinetic factors. A better measure of thermodynamic properties is obtained from the recently developed C_{sat} assay in which a preformed gel is sedimented and the concentration of the supernatant, consisting of monomers previously in equilibrium with the condensate, is obtained. C_{sat} is independent of total haemoglobin concentration and is thus a saturating concentration which reflects a phase separation[9–11]. Magdoff-Fairchild *et al.* suggest the possibility of two different condensed phases on the basis of their observation of a sharp change in the apparent van't Hoff enthalpy of gelation at 22 °C. Our studies show that C_{sat} is always markedly greater than the minimal monomer concentration needed to produce measurable aggregation, C_{agg}, and therefore the overall solution to gel equilibrium cannot be treated as a single sharp polymerisation and phase condensation C_{agg} reflects polymerisation free energy, whereas C_{sat}, although also a monomer concentration, reflects the separation into isotropic and tactoidal phases.

The endothermic enthalpy for the entropy driven process of gelation has been measured by Ross *et al.*[12] from the temperature dependences of C_{sat} and birefringence observations, and by direct calorimetry. ΔH falls from +4,000 cal mol^{-1} at 15 °C to 2,200 at 25 °C to zero at 37 °C The large associated ΔC_P is −197 cal degree mol^{-1}. Most of the enthalpy is associated with the polymerisation process, ΔH for alignment being in the neigbourhood of zero. These results are consistent with a polymerisation process dominated by hydrophobic interactions and alignment dependent on entropic forces as modelled by Minton[8]. The results of Magdoff-Fairchild *et al.*[9] show similar ΔH values and also a *p*H dependence of ΔH.

The three important physiological ligands of haemoglobin, oxygen, proton and 2,3 diphosphoglycerate (DPG) affect gelation. Thermodynamic studies (of C_{sat} and C_{agg}) show that protons above *p*H 6 8 favour gelation and that an optimum for gelation exists near *p*H 6.8[10,11,13] Protons also shorten the latent stage delay time[14] In our studies DPG, and more so inositol hexaphosphate, also favour gel formation On the other hand, under a single set of conditions resembling those in the erythrocyte, Swerdlow *et al*[15] show no effect of DPG on gelation. Kinetic studies show that DPG shortens the delay time[16]. The oxygen equilibria of dilute haemolysates of haemoglobins A and S have long been known to be indistinguishable even though haemoglobin S cells have a lower oxygen affinity than normal cells. Gill *et al.*[17] have now examined concentrated haemolysates and found that a concentration-dependent lowering of affinity exists for haemoglobin S at low saturations, associated with evidence of polymerisation by light scattering, but not at high saturations. The effect depends on the free energy change associated with polymerisation of the deoxygenated form.

In an extension of previous thermodynamic analyses, Minton[18] and Ross and Minton[19] have shown that the non-ideality arising from the excluded volume of monomeric haemoglobin in solution makes a very large contribution to thermodynamic activity at the high haemoglobin concentrations required for gelation The non-ideality as obtained from osmotic pressure data and the sedimentation equilibrium results of Williams[20] and Briehl and Ewert[13] can be well accounted for under a model in which the haemoglobin molecule in solution is treated simply as a hard, impenetrable, quasi-spherical particle. Ross *et al.* demonstrate that this non-ideality suffices to explain the effects of some non-S haemoglobins on gelation in mixtures with haemoglobin S; no direct interactions of copolymerisation are required. The non-ideality thus explains the previously observed nonspecific facilitation of gelation of haemoglobin S by the addition of haemoglobin A or F or ovalbumin[21,22] and receives further confirmation from the demonstration that isolated α and β subunits have the same effect on gelation of haemoglobins S as haemoglobin A[23]

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Ice-Age palaeo-oceanography of the Mediterranean

from N. J. Shackleton

THE CLIMAP project had as its first major goal the reconstruction of global climate at the peak of the last glaciation about 18,000 years ago. One purpose of this was pure geological excitement, another was to use the resulting reconstruction as a boundary condition for numerical general circulation models of the atmosphere (for example, Gates *Science* **191**, 1138; 1976). One of the most exciting moments in the CLIMAP project was the day we first compared the climate simulated by Gates' model for the ice age world, based on oceanographic data, with independent geological observations from the continents.

On the coarse grid-scale with which CLIMAP was forced to work the Mediterranean Sea looked rather insignificant, particularly considering the vast areas of Pacific we had to deal with, and it was at first treated rather summarily. However, it is an area of special interest to other workers, and Thiede has now presented a CLIMAP reconstruction of the Mediterranean alone (page 680 of this issue of *Nature*). The reconstruction is based on the Foraminifera. and its strength lies in the fact that it was possible to obtain a temperature calibration of the planktonic foraminiferal faunas of the Mediterranean utilising sediment samples from the North Atlantic and particularly from the Bay of Biscay This is important because the glacial faunas clearly include some that do not lie in the present range within the Mediterranean.

Glacial winter sea surface temperatures ranged from below 7 °C on the entire Spanish and French coasts to more than 19 °C on the Egyptian coast, while summer temperatures were below 13 °C along the Spanish and French coasts, rising to over 25 °C in the South-East. Thus the Western Mediterranean appears to have been very considerably cooler than it is today, although in the South-Eastern corner conditions were not greatly different from today.

This reconstruction will be received with great interest by archaeologists who are exploring man's existence around the Mediterranean during the last glaciation There is not nearly as much information as one might like preserved in some of the caves occupied around the Mediterranean for reconstructing the past environment, and a reconstruction of sea surface temperatures should provide a welcome input. One hopes that a synthesis will emerge which utilises the marine and terrestrial evidence together; at the least, it should be possible to calibrate Thiede's estimate of the seasonal temperature range by making oxygen isotopic measurements in some of the mollusc shells which prehistoric man so thoughtfully collected for our use.

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Behe and Englander[24] have extended this approach by examining the effects of various non-haemoglobin S proteins on the kinetics of gelation. They show that the delay time in the nucleation stage, formally dependent on approximately the 30th power of haemoglobin S concentration[25], is about 10th order in activity. They conclude that the size of the critical nucleus, previously considered equal to the formal order of the reaction, is composed of between 10 and 18 monomers.

Pathogenesis

Eaton *et al.*[26] and Sunshine *et al.*[27] have already proposed convincingly that the delay time in the nucleation stage, dependent on a high power of the supersaturation ratio (defined as the ratio of total haemoglobin concentration to C_{sat}), can be a major factor in the clinical development or inhibition of sickle cell crises. Presented with metastable behaviour at a number of stages, at least three phases, isotropic, tactoidal and crystalline, containing highly polymerised or condensed haemoglobin, and evidence of microscopic fibre polymorphism, we may now ask whether kinetic factors at other stages of gelation might not govern pathogenesis by facilitating formation of one or another phase and/or by bypassing the most pathogenic phase. Given this polymorphism, we also need to reconsider the classical view that the sickled erythrocyte is a membrane covered tactoid. Such an approach is implied in Minton's analysis of the phase structure of the gel and suggested also by Hermans[28] observation that the formation of tactoids in rod-like poly-γ-benzyl-L-glutamate is accompanied by a sharp decrease in viscosity. Harris and Bensusan have observed that haemoglobin S viscosity, reaching a maximum after the nucleation stage, later falls to a lower level. Since this fall apparently occurs in the period of increasing birefringence, tactoid formation here may also be associated with diminished viscosity.

Under this approach, sickle cell disease can be viewed as a disease encompassing multiple phases and phase changes. Clinical variability among different patients and the episodic nature of the disease may depend on kinetic paths and on marginal changes in free energy. Accordingly, control of these factors in the natural history of the disease may be at least as fruitful an aproach to specific treatment as the development of a covalent or even noncovalent inhibitor of gelation. In fact, as pointed out by Behe and Englander, modified haemoglobin molecules which do not copolymerise still exert a highly deleterious effect due to the large excluded volume effect.

If these many major advances permit this first described molecular disease to be seen as a disease of physical chemistry, then this view can be considered to have its origin in the work of Allison[29] which anticipated many of the modern developments. ☐

Laser annealing of semiconductors

from D. Weaire and J. I. B. Wilson

In recent years the laser has become the magic wand of the physicist and chemist, facilitating spectroscopic analysis, inducing chemical reactions, separating isotopes. Laser annealing of semiconductors is emerging as yet another exciting new application.

Annealing is the healing process by which a crystalline semiconductor, imperfectly grown or damaged in the course of introducing dopant atoms, is restored to a state of comparative crystalline perfection. This is usually achieved by holding the sample at 800–1,000 °C for 30-60 min (thermal annealing). It now seems that illumination with a laser beam can in many cases produce better results in one millionth of a second!

Credit for this discovery is generally attributed to various Russian groups who published a series of papers on laser annealing in 1975-77 (see for example, Khaibullin *et al. Sov. Phys. Semicond.* **11**, 190; 1977). In due course interest was aroused in American laboratories and there has been an avalanche of *Applied Physics Letters* on the subject during the present year.

For example, a group at Oak Ridge National Laboratory has studied the annealing of surface layers of silicon implanted with 35 KeV B^+ ions. Other American groups have examined the annealing of As^+ and Sb^+ ion implants of higher energy (100 and 150 keV) in Si, and implanted GaAs. Ion implantation is a particularly disruptive doping process in which ions are accelerated to energies sufficient to displace the host atoms from their regular lattice positions. By this means shallow, heavily doped layers are produced, but many dopant ions are left on undesirable inactive sites and a great deal of structural damage is produced. Thermal annealing repairs much of this but only laser annealing is effective in removing dislocations and stacking faults. In addition, laser annealing is more efficient in rendering implanted ions electrically active, even to levels above the normal equilibrium solid solubility limit of the dopant in the semiconductor.

Shallow p-n junctions produced by ion implantation are of particular interest for solar cells. In this case the damage due to ion implantation is responsible for a shorter minority carrier lifetime in the surface layer and hence lower efficiency, when compared with conventional diffused p-n junction cells.

Laser annealing can produce cells at least equal to conventional cells in performance, with a significant overall saving in the energy consumed during production.

It has also been shown that good ohmic contacts, difficult to produce on GaAs without decomposing the compound, can be made on GaAs wafers and epitaxial layers by laser alloying of deposited metal coatings.

Work in the USA at Bell Laboratories and Western Electric, and in Italy at the universities of Rome and Catania has demonstrated the potential of laser annealing for the recrystallisation of both polycrystalline and amorphous silicon. Amorphous Si films produced by evaporation and by implanting Si^+ ions have been recrystallised by single 50 ns ruby laser pulses. Polycrystalline Si has a recrystallisation threshold of $\sim 70\,\mathrm{MW\,cm^{-2}}$, whereas amorphous silicon requires only $45\,\mathrm{MW\,cm^{-2}}$. This is probably due to the difference in their absorption coefficients.

Any one of a variety of lasers can be used, provided that the semiconductor absorption coefficient is sufficiently high at the laser wavelength. Annealing or recrystallisation is generally limited to a 0.5 μm thick surface layer, and this means that there is no undesirable heating of lower layers which might cause unwanted impurity diffusion. Only the highest laser power levels may alter ion implanted dopant profiles, damage the surface of the semiconductor, or produce thermal stress cracks.

Such intense interest at the applied level should surely spawn more basic studies, but this does not yet seem to have happened. For example, at the recent 14th International Conference on the Physics of Semiconductors*, there was little mention of the topic, despite 'Laser Interactions with Semiconductors' as one of the themes. One obvious but difficult fundamental question which is raised by laser annealing is the following. Is it merely a thermal effect, that is, are its beneficial effects entirely attributable to brief localised heating, perhaps including melting? Alternatively, is charge carrier excitation by the ionising radiation a significant factor? Since somewhat similar results have been achieved with elec-

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*Held in Edinburgh in September, 1978.

tron beams, and since the extremely high dopant diffusivities observed are compatible with the formation of a molten region, the thermal hypothesis is strongly supported by some experiments. On the other hand, X rays have been observed to enhance the diffusion of metal contacts into the surface of semiconductors and photoionisation may enable charged interstitial impurities to move more easily to vacancies in the crystal lattice. Certainly melting is not essential for removal of ion implantation damage.

This kind of debate has already been conducted in a closely related area. The use of a laser beam to recrystallise locally a chalcogenide glass (a multicomponent amorphous semiconductor such as sputtered Ge–Te–As) was proposed several years ago as an optical storage technique. Even in this case, the relative role of thermal and photostructural effects remains obscure. □

Damn the dogma

from John C. Allen

A TOURIST hotel set in the mountains 350 km North of Oslo was the venue for a recent meeting on Chalones*, a most controversial subject in cell biology. The original hypothesis, which, alas, has been elevated to the status of a dogma, proposed that tissue homeostasis is maintained by mitotic inhibitors termed chalones, which are non-cytotoxic, tissue specific and species nonspecific, and which are secreted by the tissue over which they exert their effect. The theory has been beset by problems ever since its formulation by Bullough and Laurence in 1960, not the least being that in 18 years of intensive research there has been no confirmed report of the isolation of a pure chalone, much less any firm molecular identification. Nevertheless, the scientific importance and potential clinical uses of such substances are manifold, and the literature abounds with reports of partially purified epidermal, granulocytic and lymphocytic chalones. Certainly, the concept has stimulated an immense amount of research, and it was fitting that the participants at the meeting demonstrated their debt to William Bullough (Birkbeck College, London) by presenting him with a portrait to mark his forthcoming retirement.

The organisers had invited two eminent 'devil's advocates' from more respectable realms. A. B. Pardee (Sidney Farber Cancer Institute, Boston) and R. W. Holley (Salk Institute, San Diego) fulfilled this difficult role admirably and with unanimity. In their summing up, Pardee believed that there was no evidence for the existence of chalones as defined by Bullough, and Holley exhorted all to stop concerning themselves with restrictive definitions with the memorable phrase 'damn the dogma'. However, both felt that endogenous inhibitors do exist and that workers should now set about purifying them from associated nonspecific activity.

Indeed there was no shortage of inhibitors: over 30 were described, with molecular weights ranging from 100,000 to 300! Doubtless, several will prove to be identical, and many trivial. Among the more interesting is that painstakingly isolated from blood by W. R. Paukovits (Cancer Research Institute, University of Vienna.) This is an SH-containing pentapeptide of molecular weight 590 which apparently contains a pyroglutamyl N-terminus. It exerts a fine control over granulocyte proliferation but is inert towards thymic lymphocytes. The nearest we have to a pure lymphocyte chalone is the thymus-derived peptide of J. C. Houck (Virginia Mason Research Center, Seattle). This substance, of molecular weight 1400 and purified by final cellulose chromatography, inhibits lymphoblast formation by 50% in mixed lymphocyte culture at only 1 μg ml^{-1}. Houck informally revealed that sequencing is well under way. It can only be hoped that his characteristic optimism will this time be justified, and that a homogeneous preparation is in the offing.

Problems associated with isolation received thorough discussion. G. Isaksson-Forsen (University of Oslo) and A. Quirk (University of Newcastle) independently reported aggregation with concomitant loss of activity during purification of epidermal inhibitors. Other explanations for chalones' elusiveness were suggested, including their secretion as zymogens and their tendency to adhere to larger molecules. However, there is little firm evidence, and such effects may turn out to be 'laboratory specific'.

Unfortunately, much work reported was on impure and even crude tissue extracts. Surely, the dangers of spurious effects from artefacts should be appreciated by now? J. C. Allen (North E. Wales Institute) described work with synchronised lymphoma cells which showed how the simple molecule spermine could exert a very specific inhibitory effect. H. R. Maurer (Freie Universität, Berlin) warned of the dangers of reliance on the measurement of tritiated thymidine as sole indicator of cell proliferation, and advocated colony counting in preference. It was agreed that no single assay is satisfactory; perhaps the best plan is to adopt a simple *in vitro* screening procedure and to substantiate the results with a series of assays which must include an *in vivo* test.

Recent studies on epidermal inhibitors have provided some intriguing pointers to their true specificity and perhaps to *in vivo* function. A. Thornley (Witwatersrand University) has discovered that his epidermal chalone has an inhibitory effect not only on epidermis, but also on other specifically epithelial tissues including lung, trachea and bladder. This substance may thus be cell line, rather than tissue specific. In addition, S. Bertsch (German Cancer Research Centre, Heidelberg) has partially purified an inhibitor from a keratinised epithelial tumour of the bladder. This was active only on keratinised, and hence differentiated epithelial cells. This is evidence, albeit insubstantial, that the inhibitor was acting to maintain differentiation rather than as a cell cycle modulator.

Thus many workers have already subconsciously rejected the 'dogma' and have adopted a simple operational definition of the useful word 'chalone'. Perhaps the most encouraging aspect of the meeting was the healthy self-criticism. There is now only one reason why chalone research should not move into the mainstream of cell biology, and that is the lack of a homogeneous preparation. This sense of frustration is understandable, but the cell biologists will have to be patient and give the biochemists time to catch up. □

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*An International Symposium on Chalones in Normal and Malignant Cell Populations was held at Geilo, Norway on 29 October–2 November. It was sponsored by the Norwegian Cancer Society and was convened by Professor O. H. Iverson (Institute of Pathology, University of Oslo).

Status of biocontrol in medical entomology

OVER the past two decades, there have been major and continuing advances towards practical biological control of arthropod vectors of disease. Until the late 1950s, biocontrol in medical entomology meant little more than the (sometimes ecologically irresponsible) use of larvivorous fish—primarily *Gambusia affinis* subspp. Since then, though, the growing number of investigators in this field have increasingly concentrated upon the feasibility of control by mass-produced microorganisms and parasites.

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The current status of biocontrol of vectors is nowhere better exemplified than by its place in the World Health Organization's Special Programme for Research and Training in Tropical Diseases (TDR). This aspect of the Special Programme is emphasising innovative approaches to the control of the major vector-borne diseases of developing countries. Little more than a century ago, most of these diseases were still rife in today's industrialised countries, in some of which certain such causes of widespread morbidity and mortality (malaria in the southern United States, for example) persisted in significant foci until after World War II. During 1978, some 20 research contracts were granted under TDR's biocontrol component. About 30 will be implemented during 1979, notably in West Africa.

Hitherto, vectors of malaria, the filariases, and trypanosomiases, and arboviral diseases (likewise the snail hosts of schistosomiasis) have been attacked almost exclusively by the application of toxic chemicals. These, particularly the persistent synthetic organics, have lost popularity for various reasons including political ones. Undeniably, when over-applied, they can have damaging effects on health and environment. Moreover, genetically based resistance to them seems inevitable. Therefore, the prospects for practical biocontrol of vectors (within the broader concept of ecologically soundly based integrated control) have secured wide interest.

Applied invertebrate pathology is the aspect of biocontrol of vectors that has developed most strikingly in the past 20 years. In consequence, certain bacteria, fungi, protozoa and nematodes can now be seriously considered for widespread use against medically important arthropods. The status of this field was discussed at a recent meeting*. Two groups of microorganisms already showing promise in agricultural and forestry entomology were not dealt with—entomopathogenic viruses because not is yet ready for commercialisation in public health entomology, and protozoa because the results of a 1977 Round Table on their potential for biocontrol are about to be published in a post-congress volume from the 5th International Congress of Protozoology (New York) Relevant examples from the other major taxa follow.

Possible agents

Laboratory and field testing of the efficacy of *Bacillus thuringiensis* var. *israelensis* and *B.sphaericus* (notably strain 1593) has demonstrated high pathogenicity to larval mosquitoes, including in the case of the latter species, anophelines in Nicaragua WHO/TDR supported the work there, and is arranging for designation of a strain of *B.t.israelensis* which will shortly be tested against *Simulium damnosum* in West Africa following very encouraging tests of this bacterium against larval blackflies in Newfoundland. Both bacteria are mass-producible by available technology. So far, neither has been shown to pose health or environmental hazards, questions to be further investigated as standardised powder preparations are considered for commercial production.

Some of the fungi eligible for evaluation for vector control (for example, *Culicinomyces* of mosquitoes, *Simuliomyces* of blackflies and *Pseudocoelomomyces* of tabanids) were only recently discovered. This suggests that greater attention to this topic will inevitably reveal yet unknown fungal (and of course other) candidates. Those already under consideration range from specialised aquatics (including mosquito pathogens of the genera *Coelomomyces*, *Lagenidium* and *Leptolegnia*) through Entomophthorales (including *Entomophthora culicis* and two organisms lately described from the USSR, *Pseudocoelomomyces milkoi* and *Simuliomyces lairdi*), to Fungi Imperfecti. A representative of the latter, *Metarhizium anisopliae*, has already been tested with promising results against mosquitoes, including anophelines, in Nigeria. Similarly promising tests have been undertaken with *Culicinomyces* against larval mosquitoes in Australia.

Trials have also been carried out from the tropical South Pacific to North America and the USSR, with species of *Coelomomyces* (none of which can yet be mass-produced except *in vivo*, using laboratory colonies of mosquitoes), *Lagenidium* and *Leptolegnia* Certain of the fungi under assessment, notably *M. anisopliae* (long since cultured in large quantities and widely applied in the USSR), can also be mass-produced on simple media. This is of obvious interest to nations with restricted access to hard currencies.

Various nematode worms, primarily Mermithidae, have been identified and studied from all major vector groups To date, however, only one species—the warm-water mosquito mermithid, *Romanomermis culicivorax*—is approaching commercial production (the first registration based on it having been issued in the USA in 1976). Mermithids can be applied in the field by equipment developed for chemical pesticides, from pressurised hand-sprayers to helicopter booms. Major field tests so far undertaken have involved the use of as much as 750 kg of cultures against *Anopheles albimanus* in a land-locked lake in El Salvador, with up to 94% reduction of the host Smaller-scale tests in progress under WHO sponsorship have demonstrated the feasibility of practical applications of *R. culicivorax* against container-breeding aedine filariasis and dengue-vectors in the Republic of Western Samoa and the Tokelau Islands, South Pacific.

Commercial prospects

Turning now to imminent commercial prospects, there are two main industrial interests The first is in spore-forming bacteria, exemplified for present purposes by *Bacillus thuringiensis* var. *israelensis*, and *B. sphaericus*. Further moves in this connection will of course be much influenced by the history of lengthy investment and successful commercial production of *'B.t.'* products against agricultural and forestry pests. The second interest concerns *Romanomermis culicivorax*, in which two American companies have already shown interest.

One of these firms has stopped all research and product development work on a mermithid product for financial reasons. The second, Nutrilite Products, Inc. (NPI) is commencing its second year of work aimed at commercial development and sales.

Two major problems that still demand a solution are the design of a shipping container that will protect the mermithid eggs during storage and shipment, and elimination of fungus contamination of the cultures. A 2 year $30,000 WHO/TDR grant has just been awarded to NPI to continue work along these lines. Even if these problems are solved there is some risk that demand for this biological may not be great enough to sustain a profitable business venture. Additional market research will be needed in this area.

In sum—granted necessary encouragement, biocontrol procedures for use against arthropods of medical and veterinary importance could become a significant element in integrated methodologies for vector control in the very near future. However, they must become so, for obvious reasons of environmental unacceptibility of, and inevitable resistance to the synthetic organic pesticides. The only real problem to be faced, therefore, is that of adequate support—at a major level, and now ☐

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Clutch size in plants

from Robert M. May

THE idea that animals will tend to have smaller litters or clutch sizes in the tropics than in temperate regions has been around for some time. The underlying thinking is that environments tend to be more predictable and biological interactions to be relatively more important in the tropics (so that an evolutionary premium is placed on investing in the care of young), whereas temperate environments are relatively less predictable (so that the evolutionary pressures are to buy as many tickets as possible in what is necessarily a chancy lottery). These notions are developed more fully in, for example *Nature* **257**, 737; 1975; **267**, 394; 1977.

Much data on clutch and litter sizes of birds and mammals supports this view. Lack put together information on birds which (as summarised by Southwood *et al. Am. Nat.* **108**, 791; 1974) shows 82 species that inhabit tropical forests to have an average clutch size of 2.3, 260 species from tropical savannas to have an average of 2.7, 21 species from tropical arid areas to have 3.9—an overall average clutch size of 2.7 for 363 tropical species; in contrast, 88 species from middle Europe have clutch sizes averaging 5.6. In a related but more detailed study, Cody (*Evolution* **20**, 174; 1966) took more than 200 species of birds in the families Tyrannidae, Icteridae, Anatidae, Thraupidae-plus-Parulidae and Troglodytidae, and graphed the linear regression of clutch size against latitude for each family. The regression lines all have a roughly similar slope, corresponding to the mean clutch size increasing from around two to around five or six as one goes from the equator to 50° north or south latitude. A similar regression analysis of litter size as a function of latitude for North American mammals gives more varied results (Lord *Am Midl. Nat* **64**, 488; 1960). This work is most easily summarised by christening the slope of the regression line to be s; s then measures the increase in mean clutch size per 1° increase in latitude (Cody's results correspond to s around 0.06, with a range from 0.03 to 0.09). Lord finds statistically significant patterns of increase in litter size with latitude for 21 species of rabbits ($s=0.25$), 16 species of tree squirrels ($s=0.05$), 18 species of meadow voles ($s=0.13$), 7 species of chipmunks ($s=0.05$), 14 species of deer mice ($s=0.16$) and 12 species of shrew ($s=0.12$). Ground squirrels, pocket gophers and rats show increases that are not statistically significant, while foxes, cats, and mustelids show no apparent correlation between litter size and latitude. Tinkle, Wilbur and Tilley (*Evolution* **25**, 55; 1970) have compiled data for lizards, which show 28 tropical species having an average clutch size of 5.5 while 57 temperate species have 6.1; although in the expected direction, this difference is not statistically significant.

The broad evolutionary arguments that underline these studies of animal reproduction apply equally to plants, and it is interesting to ask whether given groups of plants manifest systematic trends in 'clutch size' as a function of latitude. Levin and Turner have recently undertaken such a study, arguing that "the number of ovules per flower (or per head in the Compositae) may be referred to as clutch size in plants, in that the development of a seed cluster from these ovules represents a discrete reproductive episode and energy commitment in time and space". (Appropriately enough, their paper is in a book commemorating David Lack: *Evolutionary Ecology*, (eds. Perrin and Stonehouse) Blackwell, 1977). One obvious problem here lies in deciding how to define 'clutch size' for plants. Levin and Turner's choice has the incidental advantage that the number of ovules per flower is relatively constant for a given species, often indeed being used by systematists as a diagnostic character. However, the net reproductive strategy for a plant also involves the total number of flowers and thence seeds, along with many other factors, which complicates any comparison with animals. Levin and Turner acknowledge this caveat, but feel their definition of plant clutch size makes for an analogy that is worth pursuing.

Levin and Turner explore the clutch size relationship within the tribe Heliantheae, which is a New World tribe of the widespread family Compositae. Their study encompasses 1,007 species from the material at the herbarium at the University of Texas at Austin. The clutch size among these species spans a wide range, from 1 in the genera *Clibadium*, *Lagascea* and *Icthyothere* to as much as 500 in other genera.

The plants are classified according to their geography (tropics, temperate, alpine) and growth form (annual herb, perennial herb, woody shrub). The average clutch size in each category is as tabulated, with the sample size in parentheses. There are consistent patterns for the herbaceous plants, and, as expected, clutch size increases with latitude. The alpine shrubs are discrepant with the general pattern; Levin and Turner offer no explanation, but it is conceivably because this growth form is under severe stress at alpine latitudes.

Growth form	Region			Form mean
	Tropics	Temperate	Alpine	
Annual herb	39(163)	62(106)	110 (1)	49
Perennial herb	61(251)	99(161)	104(34)	78
Woody	50(249)	69 (33)	45(11)	52
Region mean	52	83	90	63

Mean clutch size as a function of geographical region and growth form, for plant species in the family Compositae. The sample sizes are given in parentheses.

As each ovule may develop into a seed after being fertilised, it is also interesting to look at the weight for single seeds and the total weight of seeds per head. Levin and Turner show that the expected negative correlation between clutch size and seed weight does exist, but that it is rather fuzzy. More significant is the total head weight, obtained by multiplying the mean clutch size by the mean seed weight. This quantity, which measures the amount of energy invested per head, averages out at 560 mg for shrubs, 350 mg for perennial herbs, and 90 mg for annual herbs. Grouping the species according to geography rather than growth form, Levin and Turner find an average head weight of 300 mg for tropical species compared with 400 mg for temperate ones. This latter result is statistically significant, and accords with our preconceptions.

As emphasised above, clutch size is only part of the overall reproductive picture, which for plants must also include seed weight, total number of seeds, dispersal mechanisms and patterns, and so on. The interplay between reproductive strategies and the shapes and sizes of seeds is discussed by Harper, Lovell and Moore (*Ann. Rev. Ecol. Syst.* **1**, 327; 1970) in a masterly review that defies any glib summary. Levin and Turner survey estimates of the fraction of a plant's annual energy budget that is allocated to reproduction, and suggest for the Compositae the figure is roughly 25%

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for annual herbs, 15% for perennial herbs, and probably less than 10% for woody composites. They conclude "these general patterns suggest that, small clutch size not withstanding, annual species of the Heliantheae will have, on average, larger reproductive efforts than perennial herbs. Small clutch size in shrubs will be associated with relatively small reproductive risks in time."

In short, Levin and Turner's study demonstrates 'clutch size' differences that are related to geography and growth form. The authors argue that "there is no reason to assume the patterns observed in the Heliantheae are unique to that tribe or the Compositae as a whole", and I echo their hope that their analysis will spark similar analyses of other plant groups. ☐

Synchrotron radiation at York

from G. V. Marr and I. H. Munro

SEVEN hundred delegates, over two thirds of whom were from outside the UK assembled for the fourth general conference of the European Physical Society.* In this, its tenth year of existence, the EPS took for its theme 'Trends in Physics' and 'Synchrotron Radiation' was one of the eight parallel symposia which formed the basis for a revealing insight into the varied interests of the EPS.

Synchrotron radiation and its applications was introduced and reviewed by S. P. Kapitza (Institute for Physical Problems, Academy of Science, Moscow). This general introduction to synchrotron radiation research was followed by papers on general properties and instrumentation, atoms and molecules, photoemission from solid surfaces using synchrotron and ultraviolet sources, X-ray scattering, X-ray imaging and structural studies.

For the duration of the conference, a large exhibition had been assembled by the Daresbury Laboratory of the UK Science Research Council to describe the nature, generation and exploitation of synchrotron radiation. In addition to providing information concerning the new synchrotron radiation source under construction at Daresbury contributions of material were provided from a number of European laboratories at which facilities are available for synchrotron radiation research. Representatives were present from the laboratories at Bonn and Braunschweig (West Germany), Daresbury (UK), Frascati (Italy), Hamburg (West Germany) and Orsay (France). In addition, the conference was also attended by a group of scientists from laboratories in Moscow, Novosibirsk and Yerevan in the USSR. This group visited the UK under the auspices of the USSR Commission on Synchrotron Radiation with whom in 1977 the UK Science Research Council drew up an agreement for the exchange of information and the holding of joint seminars on synchrotron radiation research.

Reviews and reports were presented at the conference on the following topics. the use of X-ray synchrotron radiation for structural resarch in biology; recent advances in the dynamics of photoselected excited states of molecules and ions; photoemission from solids with synchrotron radiation; elastic scattering and structural applications; synchrotron radiation and X-ray crystallography; X-ray imaging, lithography, microscopy and topography; EXAFS and its use in the study of glass; atomic physics. During the meeting the contributed papers and poster contributions led to discussions of problems associated with the development of new synchrotron radiation sources and of X-ray monochromators; on the soft X-ray and VUV spectroscopy of atoms and molecules, on surface and solid state studies, on the dynamics of protein molecules, on phase transitions in biological systems, the study of clay gels, recrystallisation and grain growth, and the structural studies of powdered materials. Techniques were discussed giving rapid recording of data from these very intense X-ray sources which could be used to yield structure refinement with rapidly changing sample environment.

An unusual feature—for a physics conference—was the evident importance of synchrotron radiation research in biology and in biochemistry This was clearly demonstrated in the use of high resolution X-ray absorption spectroscopy (EXAFS) to elucidate the structure of, for example, polynuclear copper compounds which do not crystallise and of other complex metal containing proteins. Results were given on time resolved X-ray diffraction studies in the millisecond domain for muscle and on high resolution X-ray diffraction data on protein crystals, for example, tyrosyl-tRNA synthetase single crystals. Synchrotron radiation sources are intensely modulated over a wide frequency range enabling the mobility of protein molecules to be observed Using the technique of time resolved fluorescence polarisation spectroscopy in the ultraviolet region a number of proteins were shown to have widely differing degrees of rotational freedom of a single amino acid residue (trytophan). The measured amplitudes and states of these motions suggested that elementary steps of functionally significant conformational changes take place in the subnanosecond time range.

The meeting revealed to the physics community at large that synchrotron radiation sources have a major contribution to make in an astonishingly wide variety of techniques and fields of scientific endeavour. Contributions indicated that developments such as undulators and the free electron laser will become increasingly important during the next decade Indeed it is likely that future synchrotron radiation sources will be considerably different from the present generation where the radiated electromagnetic waves are the result of simple centripetal acceleration of high energy electrons confined to move on a circular orbit by a large iron core dipole magnet. The introduction of periodic magnetic structures within a straight section between conventional dipole magnets in a storage ring can result in very different forms of the emitted soft X-ray spectrum. When the vertical magnetic field alternates in direction up and down along the length of the beam orbit traversed by the electron, the oscillating electron emits a sharply peaked electromagnetic radiation spectrum This peak occurs at a wavelength related to the product of the periodicity of the field with the square of the rest mass energy of the electron and has a width which depends inversely on the number of periods in the undulator. Increases in photon flux of up to one hundred times over that of the normal synchrotron radiation spectrum are predicted for undulator peaks in the soft X-ray region. Results reported at the conference provided details of the spectral features, angular distributions of undulating radiation and polarisation which are in basic agreement with theory, demonstrating that with further development these devices can play a significant part in the next generation of synchrotron sources.

The Synchrotron Radiation Committee of the European Science Foundation, held a two-day workshop during the EPS Conference on the specification and design of a proposed new European Synchrotron Radiation Source. This will form part of a feasibility study to be considered by the European Science Foundation late in 1979 and undulators will represent a significant part of this proposal. ☐

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review article

Ogston and the development of prochirality theory

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Ogston's explanation for differentiation of identical groups in symmetrical compounds led to the important stereochemical concept of prochirality, which in turn, has made significant contributions to the understanding of enzyme reaction mechanisms.

IN 1948, Ogston postulated[1], contrary to opinions then prevailing[2], that identical groups in symmetrical compounds such as citrate and aminomalonate could be distinguished, one from another, in enzymatic reactions. He suggested a "three-point attachment" of a symmetrical substrate, $Ca'a''bc$ (the identical a groups are distinguished by different symbols only to facilitate discussion), to three binding sites, A', A'', B (which bind, respectively, a groups and the b group) on an enzyme surface. The arrangement shown for 1 (Fig. 1) is the only way in which binding can be accomplished (assuming further that binding of substrate from below the enzyme surface is not possible). It can be seen, preferably with the help of molecular models, that a' cannot be bound at site A'', with a'' at site A', since to do so requires that c bind at B. If $Ca'a''bc$ had been prepared in a stereo-specific manner so that only one of the a groups (for example, a') contained an isotopic label (●), and if only one of the A sites (for example, A'') was active in some catalytic process, a distinction could be made between the two a groups. As a result of these considerations, Ogston concluded "Asymmetrical occurrence of isotope in a product cannot be taken as conclusive evidence against its arising from a symmetrical precursor."

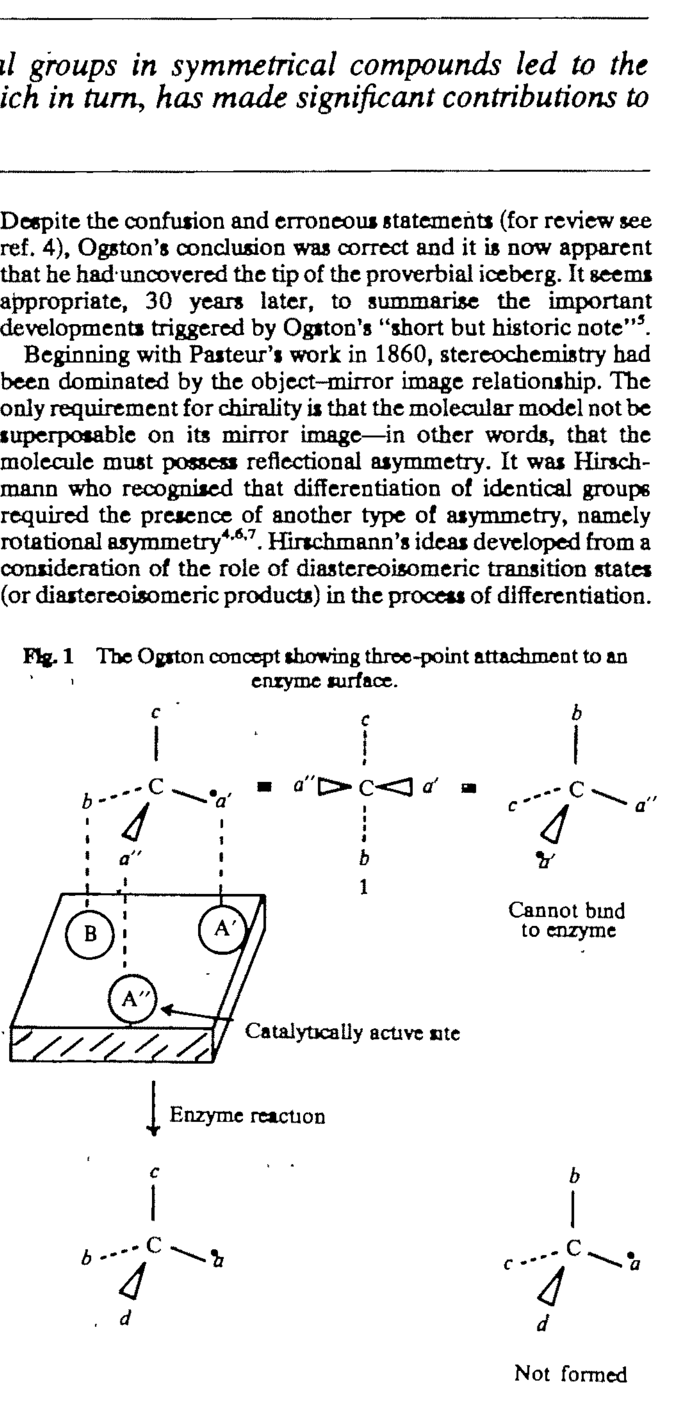
Ogston's letter stimulated lively discussion among biochemists who found it hard to accept that a symmetrical compound could behave in a non-symmetrical fashion. The difficulty people had in understanding this notion was vividly recalled by Vennesland[3]:

> "In a mood of desperation, I sat down with Ogston's paper and took some stick and ball models to assist my thinking. Ogston's paper didn't help, though I read it several times attentively. I thought he was implying a substrate might stay stuck to the enzyme, and that did not help one bit. While I was staring bleakly at the models, they suddenly told me the answer, but not in words, just in a picture. What happened in that moment of sudden insight seemed to be that I was suddenly graced with the ability to see the asymmetric carbon atom as van't Hoff had originally seen it. The feeling I experienced was a curious combination of exhilirating, sweet relief (because there was no contradiction between the two sets of experiments) and total dismay· 'Oh you idiot, why haven't you realised that before'. There were quite a few of us who had experiences similar to mine after the appearance of Ogston's paper. Even though I felt I had grasped the point quite well I found that when I tried to transmit my comprehensions to others it was like the tower of Babel. I distinctly remember hearing myself say 'Of course I know it is symmetrical, but it isn't symmetrical'."

Despite the confusion and erroneous statements (for review see ref. 4), Ogston's conclusion was correct and it is now apparent that he had uncovered the tip of the proverbial iceberg. It seems appropriate, 30 years later, to summarise the important developments triggered by Ogston's "short but historic note"[5].

Beginning with Pasteur's work in 1860, stereochemistry had been dominated by the object–mirror image relationship. The only requirement for chirality is that the molecular model not be superposable on its mirror image—in other words, that the molecule must possess reflectional asymmetry. It was Hirschmann who recognised that differentiation of identical groups required the presence of another type of asymmetry, namely rotational asymmetry[4,6,7]. Hirschmann's ideas developed from a consideration of the role of diastereoisomeric transition states (or diastereoisomeric products) in the process of differentiation.

Fig. 1 The Ogston concept showing three-point attachment to an enzyme surface.



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This role had been first recognised by Wilcox[8], and can be explained as follows. Suppose a reagent reacts with $Ca'a''bc$ in a process involving one a group. Two possible transition states (or final products) will exist, one X' for reaction at a', the other X'' for reaction at a''. If the relationship between X' and X'' is enantiomeric, the process produces equal amounts of each since enantiomers have equal stabilities and are formed at the same rate. Hence, in such conditions a' would not be distinguished from a''. If, on the other hand, X' and X'' are diastereoisomers, not in object–mirror image relationship, they are produced in unequal amounts since diastereoisomers have unequal stabilities or are formed at different rates. In these conditions, differentiation of the a groups could occur.

The recognition of the diastereoisomerism as the dominant factor in differentiation, either in the actual formation of a transition state or product, or even in the approach of a reagent, was a crucial step forward. The final proportions of products could depend ultimately either on the probability of successful collisions of reagent with a' or a'' (kinetic control) or on the relative stabilities or solubilities of products (thermodynamic control). Since enzymes contain many centres of chirality the diastereoisomer ratios in a transition state may become very high with almost complete formation of only one of the two possibilities.

Rotational asymmetry

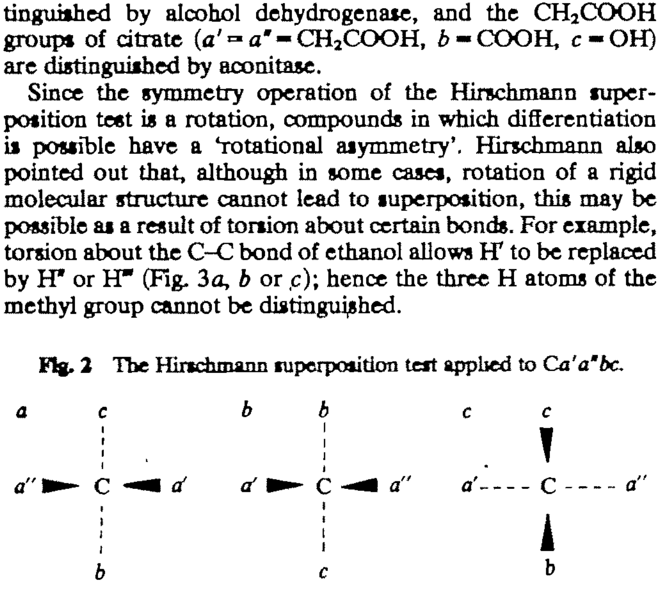
After emphasising the role of the diastereoisomeric intermediates, Hirschmann[6] provided a test to identify those structures in which differentiation of identical groups was possible (at least in principle). The role of diastereoisomeric intermediates required that "the basis for the differentiation of identical groups be sought in some structural characteristic of the substrate". The following "superposition test" was then proposed:

> "A three-dimensional representation of a molecule containing two (or more) identical groups or atoms a designated as a' and a'' is moved so that the position of a'' will coincide with the original position of a'. If this can be done in such a way that the second arrangement is indistinguishable from the first, the groups a' and a'' cannot be differentiated from each other in any reaction. However, if such superposition is impossible, the groups a' and a'' can react with an asymmetric reagent at different rates".

To illustrate the Hirschmann test, I shall consider the two a groups of $Ca'a''bc$. Attempts to rotate the model (Fig. 2a) so as to position a'' on a' lead to the arrangements (Fig. 2b and c). Neither of these rotated arrangements, however, can be superposed completely on the original (Fig. 2a). Hence, a' and a'' in this structure are potentially distinguishable. Many such differentiations have been described in enzymatic reactions (for reviews, see refs 3, 4, 9–14); to quote only two examples, the protons of ethanol ($a' = a'' = H$, $b = CH_3$, $c = OH$) are distinguished by alcohol dehydrogenase, and the CH_2COOH groups of citrate ($a' = a'' = CH_2COOH$, $b = COOH$, $c = OH$) are distinguished by aconitase.

Since the symmetry operation of the Hirschmann superposition test is a rotation, compounds in which differentiation is possible have a 'rotational asymmetry'. Hirschmann also pointed out that, although in some cases, rotation of a rigid molecular structure cannot lead to superposition, this may be possible as a result of torsion about certain bonds. For example, torsion about the C–C bond of ethanol allows H' to be replaced by H" or H‴ (Fig. 3a, b or c); hence the three H atoms of the methyl group cannot be distinguished.

Fig. 2 The Hirschmann superposition test applied to $Ca'a''bc$.



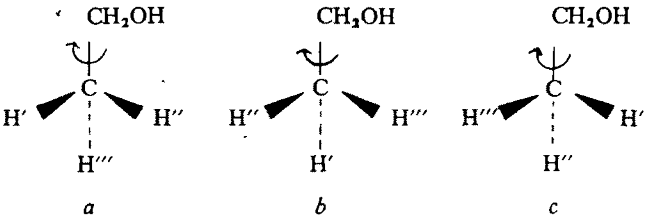


Fig. 3 Equivalence of hydrogen atoms in methyl group.

Provided that a compound has rotational asymmetry, the minimal operation which permits differentiation to occur is that of an approach of a reagent to a molecule which leads to diastereoisomeric situations for approach to a' and a''. Clearly, a "three point combination ... between the symmetrical substrate and the enzyme" is not a mandatory requirement for differentiation; Ogston has noted that his "three-point attachment" hypothesis was not meant to be taken as a literal description of the forces acting between the molecules[15]. Furthermore, identical groups can be differentiated by purely chemical reactions carried out in homogeneous solutions (for example, ref. 16). However, the three point attachment theory caught the imagination of biochemists and to this day is still quoted by some individuals as a requirement for differentiation of identical groups.

The concept of prochirality

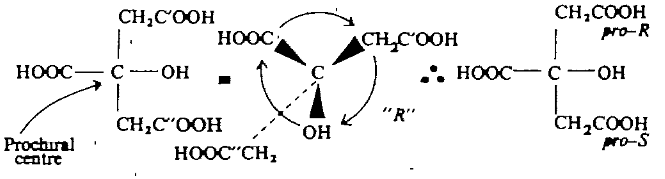
Following Hirschmann's recognition of the nature of rotational asymmetry, it became desirable to have a nomenclature system for each of the two distinguishable groups in $Caabc$. Although Hirschmann[7] had provided one solution, a more convenient system was devised by Hanson[17]. If one of the a groups of $Caabc$ is replaced by an achiral group, d, the product is the chiral compound, $Cabcd$. Since the substitution leads to chirality, Hanson coined the term "prochiral" for the structure $Caabc$. Thus, the central carbon atom of citrate is described as a prochiral centre. Furthermore, Hanson devised a prochirality nomenclature which relates directly to the Cahn–Ingold–Prelog system for specification of chirality. If one of the two identical ligands is considered to be preferred to the other by the sequence rule (without change of priority with respect to other ligands) and if the resultant priority order is R, then the (preferred) ligand is *pro-R* and the other *pro-S*. Prochirality assignment can be illustrated for citrate using the sequence priority and model shown in Fig. 4. However, for a complete description, the original paper or standard works should be consulted[9,10,17]. Although the definition, concepts, and terminology for prochirality proposed by Hanson[17] and elaborated on by Hirschmann and Hanson[18] have come into general use in biochemistry, a somewhat different approach has been advanced by Prelog and Helmchen[19]. Hirschmann and Hanson have argued persuasively for the adoption of their proposals, but clearly an international agreement would be desirable[20].

Structural relationships

An important development has been a detailed examination of the structural relationships between groups and the molecular 'environments' within which they are located. The C-1 hydrogens of ethanol, or the two CH_2COOH groups of citrate are divided by a mirror plane. The environments of these groups at their respective prochiral centres can; therefore, be described as enantiomeric. Other environments may be described in the language of isomerism. The protons at C-3 of L-serine, for example, are not bisected by a mirror plane as is the case for the two protons just considered in ethanol. The proton environments in this case are described as diastereoisomeric. The environmental relationships of the proton at C-2 and either of the protons at C-3 of L-serine would be akin to that between constitutional isomers rather than that between stereoisomers.

Isomers are now divided into three broad groups, constitutional isomers, enantiomers, and diastereoisomers (for reviews, see refs 21 and 22) by asking three questions regarding superposability. The three tests involve (1) comparison by symmetry operations of the first kind—rotation, torsion[18], and for polymers, translation; (2) comparison of bonding (connectivity) graphs (for material on graph theory and studies of isomerism see ref. 22); and (3) comparison by symmetry operations of the second kind involving reflection operations.

Figure 5 represents this series of binary tests in which the question marks signify "Superposition? yes or no", the arrows from the sides of the diamonds, yes (acceptance), and from the bottom, no (rejection). The materially identical compounds are thus compared for superposition by symmetry operations of the first kind, Sy_I (rotation, torsion); by comparison of their bonding graphs vertex by vertex, BG; and by symmetry operations of the second kind, Sy_{II} (operations involving reflection).



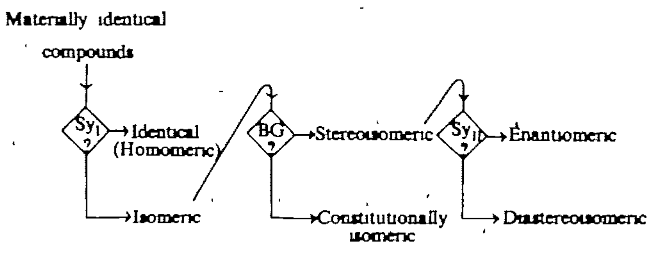
'Modified' priority sequence $OH > COOH > CH_2C'OOH > CH_2C''OOH$

Fig. 4 Prochirality nomenclature for citrate.

Hirschmann and Hanson have used a related scheme ("topic analysis") to describe environmental relationships[18,22] of materially identical groups within a molecule. Groups in identical superposable environments (for example, the three positions of H in CH_3—) are termed homotopic, those in nonsuperposable environments are heterotopic. Heterotopic (but not constitutionally heterotopic) groups are either enantiotopic or diastereotopic; these terms were first used by Mislow and Raban[23]. Enantiotopic groups are those which can be interchanged only by the formal symmetry operation S_n, that is by rotation and reflection; they reside in enantiomeric environments. Enantiotopic groups are commonly encountered in biological systems (examples are the C-1 protons of ethanol and the CH_2COOH groups of citrate). The two products formed by separate substitution of enantiotopic groups, a' and a'', by an achiral group are enantiomers. Hence a chiral reagent is required for differentiation.

Diastereotopic groups (for example, the methylene protons of citrate) are those which have the same constitution, reside in diastereoisomeric environments, and cannot be interchanged by any of the symmetry operations (rotation, reflection, rotation-reflection). There is no mirror plane between them, and if each is substituted separately by an achiral group the two products formed are diastereoisomers (this substitution test with an achiral group readily permits the distinction between enantiotopic and diastereotopic groups). In principle, diastereotopic groups can be differentiated without the intervention of any chiral agent or influence[7,24]. A diagram for topic analysis can be constructed which relates directly to that given earlier for isomer analysis; the same symbols (Sy_I, BG, Sy_{II}) are used in the topic comparisons, the operations now being applied to materially identical groups within the molecule. It should be noted that alternative classifications are being developed; Mislow has outlined a more radical classification based on an isometry on the model of the molecule[25]. As groups may be compared not only within a particular molecular structure (the topic relationships) but also in partial structures, in isolation from any actual molecule[22,25], a further extension of the binary classification system has been proposed. Comparisons of groups in isolation are termed morphic relationships; a sequence of binary congruence tests, which parallels those for isomer and topic classifications, may be easily constructed.

Fig. 5 Comparison of whole molecules—relationships between isomers.



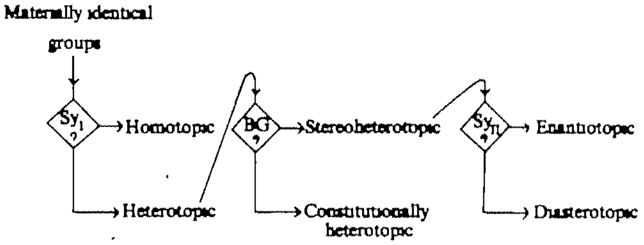


Fig. 6 Comparison of partial structures in intact molecules (or different molecules).

Retrospect and prospect

Could the concept of prochirality have been formulated before Ogston's sudden insight in 1948? Probably not. Those working in biochemistry in the period 1930–1950 tended to have either a chemical or physiological background, and in neither case were they accustomed to think in terms of the symmetry properties of molecules. As Vennesland[3] noted "Actually, anyone considering models, as Ogston obviously did, could easily grasp that compound 1 (that is, citrate with ^{14}C in one primary COOH, ^{13}C in the other) has a mirror image on which it is not superimposable. The reason this had not been grasped was that the wrong answer seemed so totally and obviously right, that no one had felt it was necessary to consult models."

It has now become commonplace to determine actual configurations of labelled compounds which have rotational asymmetry. Reviews and books should be consulted for specific examples[9–14]. One particularly interesting development has been the exploration of chirality in methyl groups containing all three isotopes of hydrogen (for review, see refs 5 and 27).

Although Ogston was primarily concerned to demolish a fallacy in the interpretation of tracer studies of intermediary metabolism, the impact of his thinking has been particularly notable in the consideration of enzyme reaction mechanisms. At a relatively unsophisticated level, the observed stereochemical course of an enzymatic reaction may allow the elimination of a proposed reaction mechanism (for examples see refs 11, 12, 14, 22 and 28). Of considerably greater interest and importance are attempts to develop more comprehensive theories, particularly by comparison of the stereochemistry of analogous enzyme reactions, and by viewing reaction stereochemistry in the context of natural selection. Hanson and Rose have suggested three important principles resulting from the natural selection for catalytic efficiency in enzymes: (1) the use of a minimal number of acidic and basic catalytic groups at the active site; (2) the maximal separation of catalytic groups and/or replacing groups; (3) the use of a minimal motion by the substrate[11,12,14,22,28]. Further research will be needed to test and refine these challenging ideas. However, the debt to Ogston is clear.

I thank Dr B. Vennesland for permission to use the quotations from her article, Drs M. L. Bentley and I. M. Campbell for helpful discussions and Drs K. R. Hanson and H. Hirschmann for suggesting the nomenclature used in the binary diagrams.

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A. G. OGSTON COMMENTS—How did I come to make my original discovery? My undergraduate training was in pure chemistry, with a strong preference for physical chemistry, and my first research was on the conductivities of dilute electrolyte solutions in Sir Harold Hartley's laboratory in Balliol. It was my interest in electrochemistry that soon led me (opportunist that I am) into the biochemical field: first with Sir Rudolph Peters, then with E. R. Holiday and J. M. Gulland, to work on the constitutions and electrochemical properties of some biological compounds; then to work (still as a physical chemist) on immune proteins, under E. R. Holiday and J. R. Marrack, at the London Hospital; finally back to Oxford for a second degree course in Physiology and to appointment at my old College (Balliol) as Tutor, mainly teaching Physiology and Biochemistry to medical students.

Some time in 1948, when spending a spare hour looking through journals (mainly with a view to advising my pupils what to read), I came across a paper[1] which seemed to show that aminomalonic acid could not be an intermediate in the conversion of serine to glycine. I read it with interest and, initially, with consent. Suddenly, something happened. One moment I thought: 'That's neat.'; the next: 'But it's wrong.'; the next: 'Citrate!' It may have taken five seconds, perhaps less. A day or two later I sent off my letter to *Nature*[2].

Why did I leave it (almost[3,4]) at that? Until I received, a few days after publication of my note, an excited letter from Sir Hans Krebs (then at Sheffield), I had little notion of the size of iceberg beneath that tip, regarding my idea as no more than an amusing piece of stereochemical logic and its consequences for asymmetric synthesis as merely an obvious corollary. And I was, by then, deeply involved with other lines of research which, if they have proved to be less sensational, have been satisfying in requiring far greater intellectual effort. 'Three-point attachment' was a gift, out of the blue, for which I have never felt able to claim much credit.

Yet I had, I suppose, unwittingly prepared myself for the three-dimensional visualisation that it required. As an undergraduate student I had enjoyed (and had been excellently taught) the intricacies of stereochemistry. During the war, on daily walks between my digs and place of work, I had amused myself by building, in my head, possible models (all quite wrong[5]) of protein structure. And I was, of course, well aware of the problem, at that time, of the place of citrate in the Krebs tricarboxylic acid cycle.

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articles

Is there a chronometer hidden deep in the Sun?

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No support is found for the conventional view of the sunspot cycle, that there exists a large random walk in the phase of the cycle. Instead, both sunspots and the [D/H] solar/terrestrial weather indicator seem to be paced by an accurate clock inside the Sun.

IT has long been believed that "the sunspot disturbances, like the eruptions of a geyser, are inherently only roughly periodic"[1]. Observations show a large variation in the ~11 yr half-cycle period, "the observed intervals ranging all the way from 7.3 to 17.1 yr"[1]. Kiepenheuer[2] has described the sunspot cycle as follows: "It was previously believed that the sunspot cycle resulted from the superposition of different periodic cycles.... Since then it has become clear that the rise and fall in the number of spots is due to a number of practically independent individual processes. Thus the idea of a true periodic phenomenon was dropped in favour of the so-called 'eruption hypothesis'. On this hypothesis, each cycle represents an independent eruption of the Sun which takes about 11 yr to die down". This conception of an irregular sunspot cycle, implying a random walk in the phase of the cycle, seems to agree with the Babcock theory and with subsequent modifications of the theory. According to the Babcock theory the poloidal magnetic field remnant of one half-cycle (~11 yr) provides the seed field

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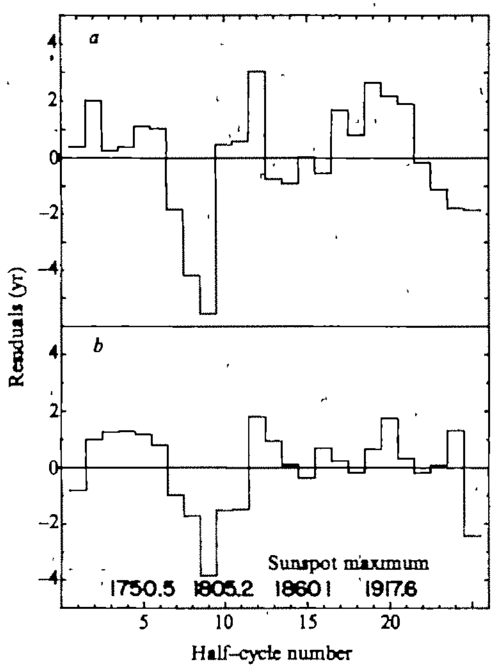

Fig. 1 *a*, Residuals (phase errors) calculated as $\delta y = y - y_0 - p(n-1)$, where y_0 and p are obtained from a least square fit of $y_0 + p(n-1)$ to y, the years of sunspot maxima. *b*, δy from equation (3).

for the generation of the toroidal magnetic field of the next half-cycle by differential winding. If, due to some vagary of the turbulent motion in the outer convection zone of the Sun, a cycle eruption should be late this lateness would be passed on to all subsequent sunspot cycles. Thus, according to the Babcock theory the sunspot cycle should undergo a random walk in phase relative to a high-precision clock.

Is a random walk in phase observed? Starting with the sunspot maximum of 1761.5 there occurred a remarkable series of three very short half-cycle periods, with an average length of 8.9 yr, after which sunspot maximum occurred ~5.6 yr too early. But this was followed by a 17.1-yr half-cycle that completely corrected the phase error. It is as though the Sun 'remembered' the correct phase for 27 yr and then suddenly reset the sunspot cycle (see Fig. 1*a*).

Statistics of the sunspot cycle

If the sunspot cycle were to be timed by some high-*Q* internal oscillator, how would we account for the remarkably large fluctuation in the intervals between sunspot maxima? One possible explanation is that the transport of magnetic field from the deep interior to the surface requires a long time and that this time interval is subject to irregularities induced by turbulence in the convective layer. In this case it might be expected that the transport interval would be lesser the greater the amount of magnetic flux floating to the surface. The phase error in the year of sunspot maximum should then correlate with the sunspot number (with a negative correlation coefficient).

To test this hypothesis the expression

$$y_0 + p(n - n_0) + r(R - \bar{R}), \text{ with } \quad n_0 = 1 \tag{1}$$

is fitted by least squares to y_n (ref. 3), the years of sunspot maxima, starting with 1705.5 ($n = 1$). Here $n = 1, \ldots, 25$, R is the sunspot number of the sunspot maximum and $\bar{R}$ is the average of the 25 values of R (ref. 3). The results are:

$$\begin{aligned} y_0 &= 1{,}704.30 \pm 0.59; \quad p = 11.136 \pm 0.042 \\ r &= -0.0397 \pm 0.0081, \end{aligned} \tag{2}$$

with an unbiased estimate of the error in y of $\sigma_y = 1.45$ yr. The validity of the least-square fit and the error estimates depends on the normality of the distribution of the residuals

$$\delta y = y - y_0 - p(n - n_0) - r(R - \bar{R}) \tag{3}$$

and their independence: δy is shown in Fig. 1*b*. The distribution is found to be approximately normal in form and the autocorrelation function shows only one (2σ) significant peak. The sunspot number estimates for the first few half-cycles may be substantially less reliable than the latter values. Omitting the first four half-cycles gives essentially the same results (equation (2)).

The coefficient r in equation (1) appears to be significant at the 4σ level. Hence we define a corrected year of the sunspot maximum by

$$\bar{y} = y - r(R - \bar{R}) \tag{4}$$

This is the best estimate of the time kept by an internal clock in the Sun, if there is such a clock.

Several statistical tests can be applied to the residuals δy (equation (3)) to help decide whether or not the fluctuations in y represent the random walk in phase required by the 'eruption hypothesis'. The first test is the number of times δy changes sign. The data (Fig. 1*b*) show 10 sign changes. A Monte Carlo calculation based on a gaussian distributed random walk in phase yields 6.1 ± 2.3 crossings, but uncorrelated gaussian fluctuations yield 12.7 ± 2.5 crossings. The independent hypothesis is slightly favoured.

The second test compares two measures of the fluctuation, the mean square residual δy_n (from equation (3)) and the mean square value of $(\delta y_n - \delta y_{n-1})$. With all 25 of the sets of sunspot data, δy_n yields the ratio 0.87 for the above two measures. With the 3σ fluctuation at $n = 9$ omitted (that is, set equal to zero) the ratio is 0.65. Assuming independent, normally distributed fluctuations in y_n, the ratio of the expectation values of the two above mean squares is $(N^2 - 1)/2(N^2 + 2N + 3)$, which for $N = 25$ is 0.46. Assuming a random walk in $\bar{y}$, namely that the error in $\bar{y}_n$ is $\sum_{m=1}^{n} W_m$ with W_m normally distributed and independent and with a uniform variance, the ratio of the expectation values of the two mean squares is $(N+2)(N^2-1)/3(5N^2+6N-3)$, which for $N = 25$ is 1.72. Again the independent fluctuation hypothesis is favoured.

Table 1 Analysis of sunspot data

n_1	n_2	y_0	p	σ
1	25	1704.30±0.55±(2.9)*	11.136±0.039±(0.32)*	1 415 (1.479)
1	13	1704.72±0.90±(2.4)	11.041±0.127±(0.49)	1.710 (1.600)
14	25	1705.52±1.66±(6.6)	11.079±0.088±(0.46)	1 055 (1.456)
5	25	1703.61±0.79±(3.4)	11.176±0.052±(0.38)	1.443 (1 572)
5	15	1703.47±1.57±(3.5)	11.178±0 165±(0.56)	1.728 (1 692)
15	25	1705.95±2.03±(7 7)	11 058±0.105±(0.51)	1 103 (1.526)

* Errors in parentheses assume random walk in phase.

The third test is more powerful. It depends on the fact that a random walk in phase implies a large expected statistical inaccuracy in the values of y_0 and p obtained from the least-square fit

$$y_0 + p(n - n_0) \rightarrow \bar{y} \tag{5}$$

The sunspot data can be analysed as a whole and also in pieces. Substantial inconsistencies would be expected in the results if a random walk in phase occurred. The results are given in Table 1. The more unreliable data are dropped in the last three rows of Table 1.

In all columns of Table 1 the first error (without parentheses) assumes independent fluctuations and the second (with parentheses) assumes a random walk. The standard deviation of the error steps W (last column, parentheses) is chosen to give the same expected r.m.s. value for $(\delta y_{n+1} - \delta y_n)$ as is obtained from the data residuals, equation (3). For independent fluctuations, σ

(last column, without parentheses) is the unbiased estimate of the standard deviation of the error in y, that is,

$$\sigma^2 = N\,\overline{\delta y^2}/(N-2) \tag{6}$$

For independent fluctuations the expected variances of the errors in p and y_0 are respectively

$$\sigma_p^2 = \sigma^2/N\overline{(n-\bar{n})^2} = 12\sigma^2/N(N^2-1) \tag{7}$$

$$\sigma_{y_0}^2 = \sigma_p^2\overline{(n-n_0)^2} = 2\sigma^2(2N-1)/N(N+1) \tag{8}$$

(assuming $n_0 = 1$).

For random walk fluctuations the corresponding expectation values are

$$\sigma_p^2 = 1.2(N^2+1)\sigma^2/N(N^2-1) \tag{9}$$

and

$$\sigma_{y_0}^2 = [(N+1)(2N+1)/6N - \overline{(n-n_0)} + 6(N^2+1)\overline{(n-n_0)}^2/5N(N^2-1)]\sigma^2 \tag{10}$$

$$\sigma_{y_0}^2 = 2\sigma^2(N^3+4N^2+11N-1)/15N(N+1)$$

(assuming $n_0 = 1$).

Table 1 shows that the agreement among the values of y_0 and p is very good, better than would be expected if there were a random walk in phase. But it should be emphasised that all the determinations of y_0 and p in Table 1 are not independent. Also, in line 3, σ_{y_0} is the expected error due to the data (14, 25). But the random-walk phase error at the termination of (1, 13) is the starting error for (14, 25).

A fourth test of the random-walk hypothesis is provided by the unbiased estimate of σ^2, given by equation (6). For a data string broken into m equal pieces the average of the m values of σ^2 should closely approximate σ^2 for the unbroken string, assuming independent fluctuations. With a random walk, the expected mean variance is a factor $(N+2m)/m(N+2)$ smaller. The r.m.s. value of σ from lines 2 and 3 of Table 1 is 1.421 yr, to be compared with 1.415 yr. From the 5th and 6th lines 1.450 yr is obtained, to be compared with 1.443. With random walk errors, 1.044 yr and 1.066 yr would be expected.

All four tests tend to support the independent fluctuation hypothesis, but this support for the 'solar clock' hypothesis is not decisive as the data sample is small and the results could represent a statistical fluke.

Pre-Maunder-minimum sunspot cycle

As a result of Eddy's work[4] it now seems to be well established that very few sunspots were observed from 1645 until 1700. An interesting question concerns the phase and frequency of the pre-minimum sunspot cycle. Was there an internal clock running while the sunspot cycle was substantially turned off?

The telescopic observations during the early seventeenth century were too few to answer this question conclusively. Eddy has found evidence for three sunspot maxima between 1610 and 1645. Taking the sunspot numbers estimated by Eddy at face value, and assuming that $p = 11.136$, gives $y_0 = 1702.2$, with the correction in equation (4). If it is assumed that R was underestimated by a factor of 2, $y_0 = 1703.7$ is obtained. The agreement of these values with y_0 on the first line of Table 1 may mean that a solar clock was running while the surface indications of the cycle were substantially switched off, but the data are too few to provide strong support for this conclusion.

A very long, reliable data base is preferable for an independent analysis of the pre-Maunder-minimum sunspot cycle. The naked-eye sunspot observations of the past 2,000 yr provide one possibility for such an analysis[5]. Unfortunately these large sunspot groups do not always occur at the time of the sunspot maximum and the resulting scatter in epoch seems to be so large as to make these data worthless. Another approach is to use the historical accounts of auroras which are known to correlate with the sunspot cycle. Again there seems to be a large phase uncertainty. There is also an additional uncertainty, that of identification. "Auroras" in ancient Far Eastern accounts are described as "dark vapours", "white vapours", "greyish clouds", and so on.

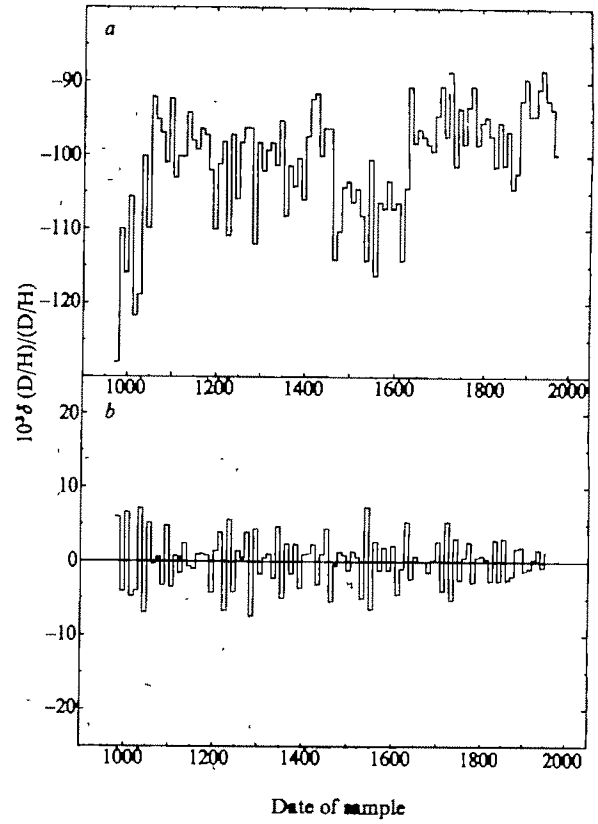

Fig. 2 *a*, Epstein–Yapp[7] [D/H] bristle cone pine data, D. *b*, Epstein–Yapp[7] [D/H] data filtered by subtracting a moving mean $\bar{D}_n = (D_{n-1}+2D_n+D_{n+1})/4$ to give $\delta D_n = D_n - \bar{D}_n$.

The long-debated hypothetical relationship between sunspots and weather might provide the necessary data. This has been a controversial matter: some atmospheric scientists say that the miniscule change in the solar spectrum asociated with the sunspot cycle should not have a substantial effect on the weather, others find evidence for a 22-yr cycle in the weather, and some astrophysicists say that a significant variation in the solar luminosity with the sunspot cycle could not occur. In discussing the 22-yr period in the drought record for the High Plains, Roberts[6] remarked in 1973 that "it will be extremely interesting to see what happens in this region in the period 1974 to 1978". Perhaps this anticipation of the drought of 1976–77 should have been taken seriously. Such a prediction could reasonably have been based on the statistics of previous droughts. The drought years of the nineteenth and twentieth centuries in the High Plains seem to be rather well-established and are: $y = 1823$, 1843, 1868, 1890, 1913, 1934 and 1954 (ref. 6). A least square fit of the form of equation (5) gives

$$\begin{aligned} y_0 &= 1889.3 \pm 0.6 \text{ y} \\ p &= 22.14 \pm 0.28 \text{ y} \\ \sigma_y &= 1.474 \text{ y.} \end{aligned} \tag{11}$$

The expected epoch of the 1975–76 drought is $y = 1977.9 \pm 1.7$. The value of p in equation (11) is in good agreement with twice the values of p in Table 1. If we assume that these periods are the same and adopt the more accurate value $p = 2 \times 11.136$ yr as the cycle period (Table 1, line 1), we obtain $y_0 = 1889.3 \pm 0.5$ and $\sigma_y = 1.375$. The drought epoch closest to 1705 is 1711.1, at the time of the sunspot minimum.

The δ[D/H] indicator

The data from which conclusions can be drawn concerning the phase and frequency stability of the pre-Maunder sunspot cycle are given in Fig. 3*a* of ref. 7. This shows the sequential variations in the deuterium/hydrogen ([D/H]) ratio of a set of 10-yr samples of cellulose nitrate obtained from two bristle cone pines that cover 1,000 yr of time without gaps. A 60-yr interval of the 1,000-yr span overlaps data from the two trees and gives consistent results[8]. For conciseness I shall use D to designate the δ[D/H] indicator, the fractional variation in the [D/H] ratio relative to [D/H] of a standard mean ocean water. Expressed in parts per thousand, this indicator is $D = 10^3\,\delta[D/H]/[D/H]$.

Yapp and Epstein[9] have noted that the [D/H] ratio found in precipitated water varies with both atmospheric temperature and mean surface oceanic temperature, and they have demonstrated that such a variation shows up in the [D/H] ratio found in the cellulose of plants that incorporate the precipitated water during their growth. The variation of D with atmospheric temperature, expressed as a mean slope is $\langle dD/dT\rangle \approx 5.4\ \mathrm{deg}^{-1}$ (see Fig. 10 of ref. 9). Epstein and Yapp[7] have demonstrated a connection between their indicator and the weather. The missing element is a convincing argument that this indicator is related to the solar cycle.

Figure 2*a* is Fig. 3*a* of ref. 7 replotted. The approximately 20-yr period is clearly visible. The power spectrum of D shows a strong peak with a 22 yr period (Fig. 4 of ref. 7). This analysis is repeated here at a higher resolution and after filtering D by subtracting a moving mean $\bar{D}$. δD is shown in Fig. 2*b*. Figure 3 shows the power spectrum of δD. The principal peak occurs at an interpolated frequency of $0.04472\ \mathrm{yr}^{-1}$ and the corresponding half-period is 11.181 yr. The agreement with p in the first line of Table 1 is excellent. This close agreement and the narrowness of the peak in Fig. 3 provide substantial support for the argument that there is a causal relationship between the sunspot cycle and the [D/H] indicator, and therefore with the weather. The spectral peak has the same width at half maximum as that of a sinusoidal oscillation observed for 1,000 yr. The existence of a purely terrestrial meteorological resonance of such high Q seems very unlikely. For the resonance frequency to accidentally agree with the sunspot cycle frequency with a 1σ accuracy of 0.5% seems equally unlikely.

Because of aliasing by the 10-yr sampling period the above interpretation of the frequency of the spectral peak is not unique. However, filtering by the 10-yr average substantially eliminates all but one of the alternative frequencies ($0.05528\ \mathrm{yr}^{-1}$) which does not seem to be the correct interpretation.

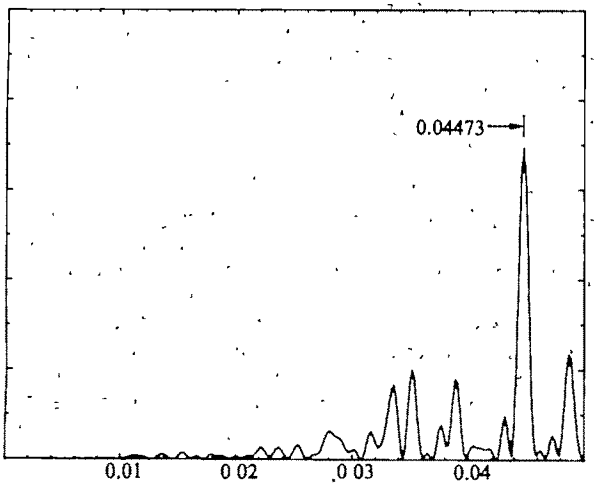

Fig. 3 The power spectrum of δD of Fig. 2. the main peak occurs at the solar cycle frequency (given by the top line of Table 1) to the statistical accuracy of the latter.

Additional support for the assumption that the Epstein–Yapp data provide information about the solar cycle is found in the apparent "finger-print" of the Maunder minimum in the data. These data (Fig. 2*a*) show a pronounced step upward in D after 1625, shortly before the start of the Maunder minimum. The cause of the step is unknown but cannot be due to the change in sample source (change in trees); conceivably it might be related to the Maunder minimum (S. Epstein, personal communication). At 1645 there occurs the start of a 50-yr interval (during the Maunder minimum) with very little variation in D, the only interval of that type in the 1,000-yr span. This quiet interval ends in 1695, just before the return of normal sunspot activity.

We shall tentatively assume a causal relationship between the solar cycle and the weather and treat the Epstein–Yapp data as a solar-cycle indicator. It should be emphasised that this does not imply that the sunspots or other surface manifestations of solar activity are directly responsible for the variation in [D/H]. And the phase of the [D/H] variation need not correspond with the phase of the spot eruption. As noted above the prominent spectral peak found in Fig. 3 is characteristic of a sinusoidal oscillation. The small, narrow secondary peaks of various heights are characteristic of a contamination of the signal by gaussian noise. If random-walk fluctuations in phase should occur the spectral character of the signal would be expected to be significantly different from the peak in Fig. 3. A complex multiple peak resonance with several peaks rising out of a broad low base is normally expected. This is illustrated in Fig. 4.

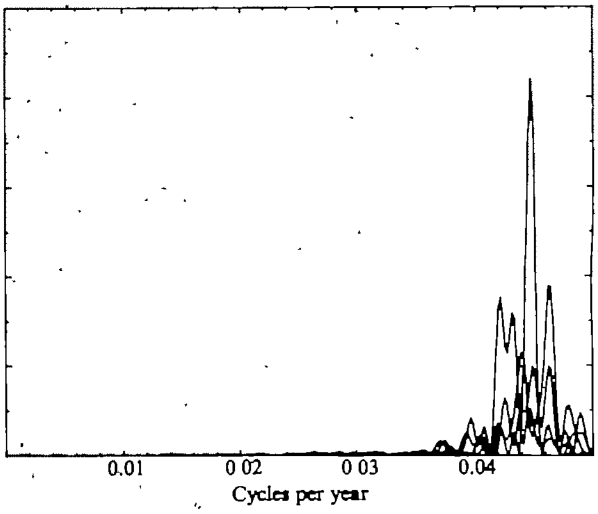

Fig. 4 The power spectra of 'dummy data', computed assuming a constant amplitude oscillation of frequency $0.04473\ \mathrm{yr}^{-1}$ and a random walk in phase. The variance of the random-walk steps $(1.402)^2/10\ \mathrm{yr}$ is equivalent to the random-walk variance $(1.479)^2$ in Table 1. Four randomly chosen examples are superimposed.

We can now examine the Epstein–Yapp data for frequency and phase stability. From equations (7)–(10) the difference between the errors expected under the eruption hypothesis and the solar clock hypothesis are more extreme the longer the data span, the error ratio being approximately proportional to the length of the span. To examine the frequency stability the frequency $\nu = \omega/2\pi$ and the coefficients c_0, c_1 and c_2 are adjusted by least squares in the fit

$$c_0 + c_1 \cos \omega(t - t_0) + c_2 \sin \omega(t - t_0) \rightarrow \delta D \qquad (12)$$

(assuming independent fluctuations) where $t_0 = 1705$. This gives $\nu = 0.044722 \pm 0.000082\ \mathrm{yr}^{-1}$ for the full data set ($n_1 = 1$, $n_2 = 98$) and a corresponding half-period of 11.180 ± 0.021 yr. (Compare with Table 1, first line.) For the first half of the data ($n_1 = 1$, $n_2 = 49$) we obtain $\nu = 0.04500 \pm 0.00019\ \mathrm{yr}^{-1}$ and for the second half $\nu = 0.04429 \pm 0.00023\ \mathrm{yr}^{-1}$. For the intervals (1, 25), (25, 49), (50, 74) and (74, 98) the frequencies are 0.04443 ± 0.00062, 0.04585 ± 0.00040, 0.04455 ± 0.00053 and $0.04415 \pm 0.00070\ \mathrm{yr}^{-1}$ respectively.

Table 2 Analysis of [D/H] data

n_1	n_2	c_1	c_2	σ_D	δ
1	98	1.11 ± 0.39	-2.47 ± 0.38	2.687	$-66° \pm 8°$
1	49	1.20 ± 0.62	-3.02 ± 0.60	3.011	$-68° \pm 11°$
50	98	1.00 ± 0.48	-1.91 ± 0.47	2.344	$-62° \pm 13°$
24	73	1.01 ± 0.53	-2.84 ± 0.55	2.683	$-70° \pm 10°$
1	33	1.85 ± 0.85	-3.06 ± 0.79	3.308	$-59° \pm 13°$
34	66	0.27 ± 0.59	-3.18 ± 0.64	2.476	$-85° \pm 11°$
66	98	1.52 ± 0.53	-1.46 ± 0.49	2.059	$-44° \pm 14°$
1	25	1.99 ± 1.01	-2.77 ± 0.96	3.412	$-54° \pm 16°$
25	49	0.87 ± 0.80	-3.31 ± 0.83	3.425	$-75° \pm 13°$
50	74	0.93 ± 0.69	-2.61 ± 0.73	2.774	$-70° \pm 15°$
74	98	1.40 ± 0.66	-1.32 ± 0.61	1.926	$-43° \pm 18°$

Adopted frequency, $\nu = 0.044722$ y^{-1}.

The agreement of the various values of ν with each other is much better than would be expected with a random walk in phase. Despite the slightly high frequency for the (25, 49) interval, the frequency determinations are internally consistent assuming independent fluctuations. To test for phase stability we adopt $\nu = 0.044722$ yr^{-1} and obtain the results shown in Table 2. In Table 2 the last column is the phase lag (in degrees) of the oscillation (relative to the time zero 1705). (A phase angle of $\delta = -66°$ corresponds to a maximum in δD at 1701.) The amount of stability of phase shown in Table 2 is consistent with uncorrelated fluctuations and is greater than that expected under the random-walk hypothesis.

The Epstein–Yapp data are consistent with the hypothesis that the solar cycle persisted through the Maunder minimum. Additional support for this conclusion is found by Mitchell and Stockton in the tree-ring data[10]. It is found that the power spectra of indices derived from the variations in the widths of successive tree rings exhibit strong but broad 22-yr peaks which seem to be due to the solar cycle[11].

Conclusions

From this it may be concluded that: (1) the sunspot cycle shows no statistical indication of a random walk in phase; (2) it appears to exhibit large fluctuations in phase relative to some internal oscillator in the Sun; (3) the large magnitude of some of the phase errors (as great as 5.6 yr) suggests that there may be a long delay in the transport of magnetic field to the surface of the Sun from the interior; (4) there is a good correlation of this delay interval with the sunspot number and presumably with the amount of magnetic flux; (5) there are several indications that the solar cycle persists through the Maunder minimum. The signal strength and the precision of the 22.36 ± 0.04-yr period seen in the Epstein–Yapp [D/H] data, the narrowness of the spectral peak and the tight agreement with the 22.27 ± 0.08-yr magnetic sunspot period strongly support the hypothetical connection between weather and the solar cycle.

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A Glacial Mediterranean

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Glacial Mediterranean palaeo-oceanography has been reconstructed from planktonic foraminifers once living in this small ocean. Inferred sea surface temperatures ranged from 13 °C in the Alboran Sea to 18 °C in the Levantine Sea during the winter and from 19 to 26 °C respectively during the summer. An influx of cool, fresh surface water from the Aegean Sea disturbed this gradient in the eastern Mediterranean.

THE Mediterranean is a small elongate ocean basin separating dry, arid and hot North Africa from humid to semiarid, temperate to subtropical Europe, and is therefore a sensitive climatic boundary at present. As a marine basin it is subdivided by shallow sills into a series of deep troughs (Fig. 1), and exchanges its water masses with the Atlantic Ocean through the Strait of Gibraltar, to a minor degree with the Black Sea through the Bosporus and with the Red Sea through the Suez Canal. During the Quaternary the pathways between the various basins of the Mediterranean, and the connection to the Atlantic, must frequently have been much narrower and more restricted than at present due to the eustatic lowering of the Glacial sea levels at the same time as the Black Sea became a fresh water or saline lake[1]. The mode of water exchange through the Strait of Gibraltar is largely climatically controlled[2]: North-east Atlantic surface waters which are relatively cool and of normal oceanic salinity, flow into the Mediterranean where evaporation exceeds precipitation and fresh water input from the various rivers. The excess inflow is partly balanced by outflowing Mediterranean subsurface waters which are dense due to their high salinity and relatively warm. They spill over the sill at Gibraltar (330 m), sink to a water depth interval[3] of ~500–1,500 m and can be traced as a separate intermediate water mass over wide regions of the Atlantic Ocean. Any change of this balance which might have occurred during the climatic pulsations of the late Quaternary, must have had important implications for the vertical water mass structure of the entire North Atlantic Ocean. There is an intense dispute about the possibility of a reversed current regime in the Strait of Gibraltar during Glacial episodes[4,5].

Mediterranean planktonic foraminifers

Here I use planktonic foraminiferal assemblages to reconstruct the Glacial palaeo-oceanography of the Mediterranean. Samples have been dated by various methods (litho- and biostratigraphy, oxygen isotope ratios, radiocarbon dating) and will be discussed elsewhere together with a detailed account of the foraminiferal data. The most important recent advance in the stratigraphy of late Quaternary Mediterranean pelagic sediments, which enabled the correlation of these sediments to the contemporaneous record in other oceans[6], is the recognition of the coincidence of the elevated oxygen isotope ratios marking the last Glacial maximum with the occurrence of the coolest planktonic foraminiferal faunas[7–9]. Glacial core levels have been sampled and planktonic foraminiferal taphocoenoses have been counted at all locations (Fig. 2*a* and *b*) except the position off Israel where published data have been used[10]. Faunal counts

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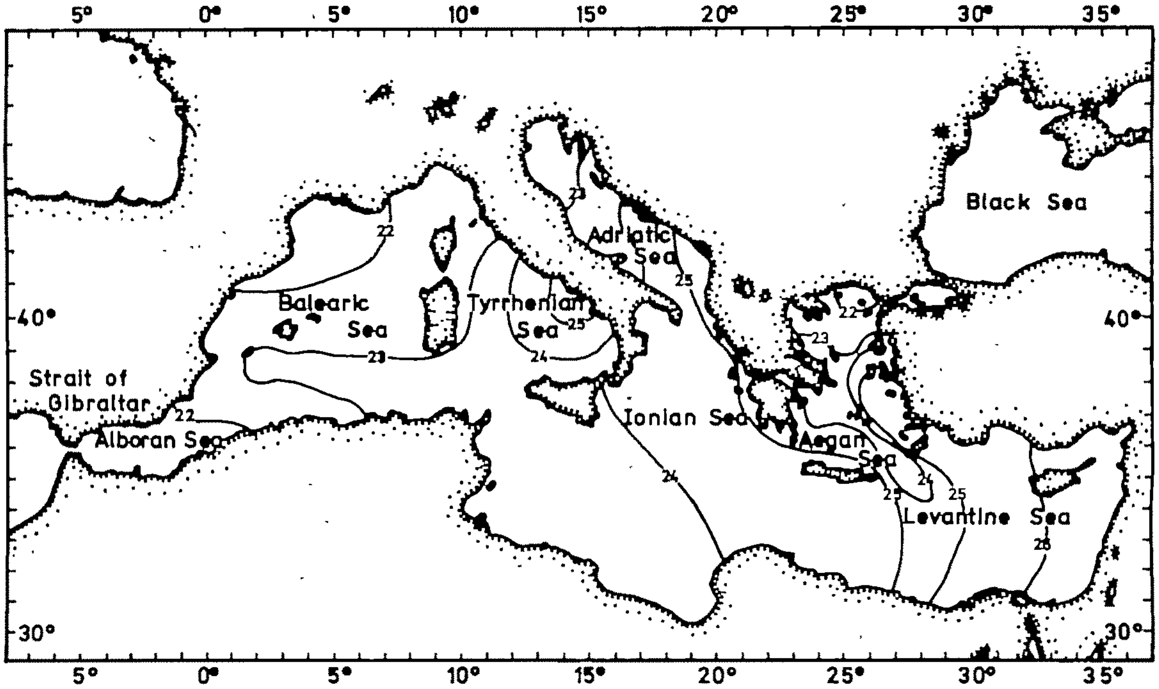

Fig. 1 Modern summer (August) sea surface temperatures in the Mediterranean after Bruns[40] and names of major basins as referred to in the text.

and palaeo-oceanographic estimates of 33 locations provide the data base for this reconstruction of the Glacial Mediterranean.

Planktonic foraminifers and other planktonic fossils have been used successfully in many parts of the world ocean[11] for palaeo-oceanographic reconstructions, but these methods have encountered difficulties when applied to regions close to the continents[12] and in marginal basins. The late Quaternary foraminiferal faunas from the East and West Mediterranean have frequently been described since the Swedish Deep Sea Expedition assembled the first substantial series of sediment cores from this region[13,14]. However, most of these studies[15] have looked at the late Quaternary fluctuations as a series of events affecting the depositional regime at a particular core location. In this study, emphasis is placed on drawing a regional, quasi-synoptic picture of the palaeo-oceanography of the entire Mediterranean Sea during the last Glacial by collecting as many samples as possible from cores where I could confidently identify this stratigraphic interval, and by using Glacial planktonic foraminiferal faunas to define the distribution of important surface water masses during the 18,000 yr BP time slice.

Atlantic surface waters transport planktonic foraminifers into the Mediterranean[16], where rich faunas dwell in the surface water masses above all the central troughs. Planktonic foraminifers, which inhabit the modern oceans from polar to tropical latitudes[17], are confined to water masses with a relatively narrow range of salinities. They cannot therefore survive or establish autochthonous faunas in marginal seas such as the North Sea or Baltic Sea[18,19] where the seawater is considerably less saline than in the open ocean, or in the Persian Gulf, where high salinities prevent the establishment of autochthonous faunas (although living planktonic foraminifers drift into this marginal basin from the Indian Ocean)[20,21]. It is therefore important that planktonic foraminifers inhabited the Mediterranean throughout the late Quaternary[22,23] despite evidence from the surrounding land masses suggesting major climatic fluctuations in this region[24,25]. The common presence of these fossils in the Glacial and Interglacial Mediterranean sediments, despite the periodically restricted pathways between the North-east Atlantic and the Alboran Sea, and between the west and east basins is remarkable because it implies that it was the Mediterranean sea surface temperature regime that responded to these climatic fluctuations rather than the salinities, although detailed investigations of the oxygen isotope ratios of planktonic foraminifers also suggest important variations of sea surface salinities[26]. The western basins are now populated by tropical, subtropical and temperate faunas while tropical faunas prevail in the East Mediterranean. A typical warm water representative of these faunas is *Globigerinoides ruber* which is a frequent component of Tyrrhenian Sea, Adriatic Sea and East Mediterranean surface sediments[27,28]. During the last Glacial this and other tropical species were restricted almost entirely to the Ionian and Levantine Seas[10,13,14,22].

Although the Balearic Sea is situated just south of a region of major alpine glaciation during the last ice age, and although we know that the Bay of Biscay was filled with polar water masses during this period[12,29], it was surprising that the typical planktonic foraminiferal marker species of polar water masses, the left-coiling phenotype of *Globoquadrina pachyderma*, never became abundant in the Mediterranean except in a small area of the Tyrrhenian basin. In the Glacial North-east Atlantic, water masses with this species have been traced as part of the eastern boundary current system along the Iberian continental margin, but they do not seem to have protruded southwards beyond the south-west tip of the Iberian peninsula. Cool to temperate species inhabited the Glacial West Mediterranean with species such as *Globigerina bulloides*, *G. quinqueloba*, *G. pachyderma* (right) and *Globorotalia inflata* being typical faunal components. Note that some of these species have also occurred in considerable abundance in the northern parts of the Levantine and Ionian Seas as well as in the Adriatic[30].

Glacial sea-surface temperatures in the Mediterranean

Although Mediterranean faunas posed a considerable problem in early attempts at synoptic palaeo-oceanographic reconstructions, my studies apparently revealed many similarities to equivalent Atlantic faunas. I have therefore attempted to explain the composition of the Mediterranean faunas statistically in terms of North Atlantic assemblages and to apply transfer functions[31] which had been developed to describe quantitatively the North-east Atlantic late Quaternary palaeo-oceanography[12], to Glacial Mediterranean planktonic foraminiferal

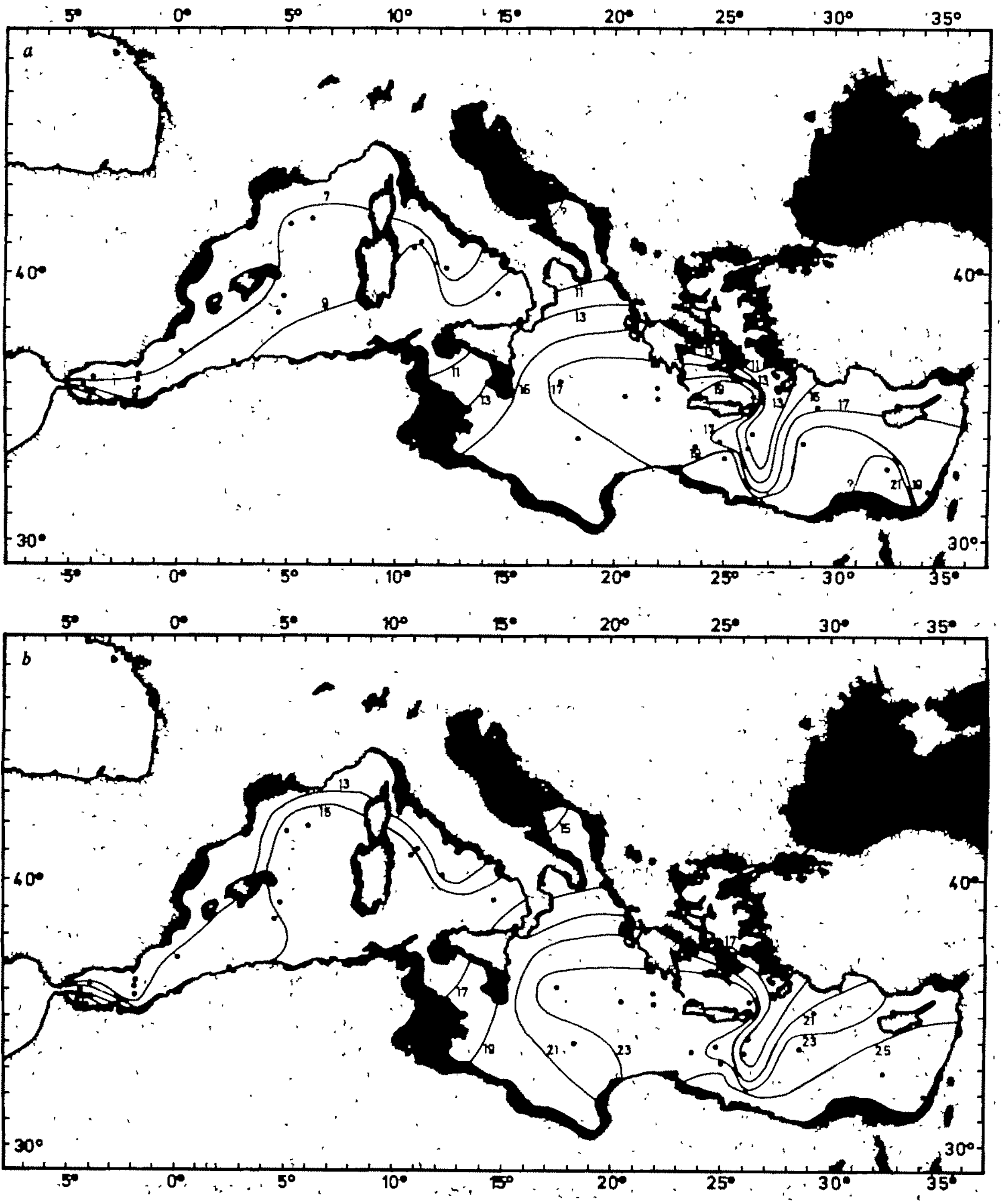

Fig. 2 Sea-surface temperatures in the Mediterranean during the peak of the last Glacial ~18,000 yr ago. *a*, During the Glacial winter (February). *b*, During the Glacial summer (August). Dots mark the positions from where Glacial core levels have been obtained. The blackened areas mark schematically changes of the Mediterranean coast lines due to the lowered Glacial sea levels.

shell assemblages. For this the data base which had been developed for the North Atlatic[32], has had to be modified by selecting the data from the North-east Atlantic and by adding a considerable number of sample points from the Bay of Biscay and from the Ibero–Moroccan continental margins[12]. These modifications were intended to recognise and allow for the fact that it is the surface waters of the North-east Atlantic eastern boundary current sustem that are diverted into the Mediterranean. Downcore studies suggest there is good reason to believe that the Glacial mode of water exchange between the Mediterranean and the North-east Atlantic resembled the modern current regime in the Strait of Gibraltar[5] when the >300 m deep strait was probably only half as deep than at present[33]. The Mediterranean can also be considered as a small ocean basin with plankton assemblages slightly different from those of the open pelagic environment, because they are affected by the hydrography of epicontinental water masses which are never very far away in this small ocean.

Otherwise the evaluation of the micropalaeontological data has followed established procedures[31] which allowed me to estimate quantitative values for past oceanographic variables. They combine Q-mode factor analysis with regression and classification analysis. The Q-mode factor analysis with the program CABFAC[34] produces two matrices. The varimax factor matrix defines the importance of North-east Atlantic planktonic foraminiferal assemblages – factors at each sample location[12], a matrix of factor scores indicates the contribution of each category = planktonic foraminiferal species to each factor. The relationship among assemblages and hydrographic variables has been established by multiple regression analysis, in this case North-east Atlantic summer (August) and winter (February) sea-surface temperatures. The resulting transfer functions have recently been used to reconstruct the Glacial North-east Atlantic[12]. In this study classification analysis has been applied to project Glacial Mediterranean downcore samples into the model derived from the modern North-east Atlantic. This step allows the factor loadings to be estimated on the basis of the predefined matrix of factor scores in each of the Mediterranean samples. Finally, these estimated factor loadings have been fed into the equations obtained by regression analysis, to calculate values of summer and winter sea-surface temperatures in the Glacial Mediterranean.

The degree to which planktonic foraminiferal faunas in each sample are explained by the Q-mode factor model, is expressed as communality both in factor and classification analysis. Thus, the calculated communalities of the Glacial Mediterranean faunas can tell how successfully they are explained by the North-east Atlantic surface assemblages. Several stations have been found where the faunas have not been explained satisfactorily by the model applied, as indicated by low communalities (cut off point in general at 0.7). These stations are located mainly in the marginal basins along the northern shores of the Mediterranean, for example in the Adriatic Sea. I conclude, therefore, that these faunas do not have an analogue in the modern North-east Atlantic. Other samples with low communalities have been observed in regions where frequent turbidites (for example, in the abyssal plains) suggest the contribution of displaced sediment components. Samples with low communalities have therefore been eliminated from further analysis.

This attempt to reconstruct the palaeo-oceanography of the Glacial Mediterranean reveals several other deficiences. Although sea-surface temperatures have been compared here with Atlantic data, other hydrographic variables might have been more appropriate. Distortions of the oxygen isotope ratios of planktonic foraminiferal shell material suggest important changes of sea surface salinities[26]. The eastern Mediterranean is today characterised by seasonal differences of temperature which are higher than those in the North-east Atlantic, because the influence of the continental climate over the adjacent land masses has an important impact on the temperature regime. The model applied here might therefore be incapable of generating sufficiently high seasonalities for the Glacial Eastern Mediterranean.

However, there are enough cores whose faunas are explained very well by the above described model (Fig. 2). The factor loadings which have been estimated in the classification analysis-step, reveal a simple regional distribution pattern of planktonic foraminiferal assemblages in the Glacial Mediterranean. Two relatively cool assemblages are concentrated in the north-west basins, an intermediate one occurs as a belt across the central part while the tropical assemblage is confined to its south-west region. Polar assemblages are virtually missing from the entire basin.

The results of attempts to contour the temperature estimates for the Glacial winter (February) and summer (August) are given in Fig. 2*a* and *b*. The temperature range spanned from 7 °C in the north-west basin to >20 °C in the south-east basin during the Glacial winter. While the temperature increased eastwards in a fairly regular fashion in the Balearic basin, the pool of warm surface waters in the Ionian and Levantine basins reveals a considerable structure because of the disruptive intrusion of a cool surface water mass from the Aegean Sea. During the summer the entire Mediterranean apparently warmed up considerably, but the basic pattern of surface-water temperatures was still preserved. The steepest temperature gradients are found close to the connection between the East and West Mediterranean basins[35], but the incursion of the cool surface water from the Aegean basin is also visible during the Glacial summer. Comparison between the modern (Fig. 1) and Glacial (Fig. 2*b*) sea-surface temperature shows how and where the most drastic temperature changes from the Glacial to the modern situation have occurred. The similarity of the pattern, although not of the absolute temperature values south of the Aegean Sea, is particularly noteworthy. The temperatures in the Glacial Alboran Sea compare well to those of the adjacent North-east Atlantic. The Bay of Biscay[12] was probably a few degrees colder than the North-west Mediterranean during Glacial times. Although we know that ice-rafted debris can be traced in the Glacial levels of cores along the north-west African continental margin as far as South Morocco[36], coarse ice-rafted terrigenous detritus has not been found in the corresponding samples from the Western Mediterranean. The higher sea-surface temperatures in the Glacial Balearic basin are also supported by the virtual absence of the polar planktonic foraminifer *G. pachyderma*.

A source for the water mass protruding from the Aegean into the Levantine basin deserves further attention. Previously it had been assumed that the fresh water influx was due to high discharge rates of the Nile river[9], but the pattern which has now been established by means of foraminiferal studies, strongly suggests a northern source[37,38]. It is commonly believed that the land areas north of the Glacial Mediterranean were much drier than at present[24,39]. If this is so, the source for this important influx of cool and probably comparatively fresh water[26] into the Aegean Sea has to be sought in the hinterland of the Glacial Black Sea which as a lake was overflowing into the Mediterranean[37,38].

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The regulatory region of the *trp* operon of *Serratia marcescens*

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The nucleotide sequence of the regulatory region of the trp *operon of* Serratia marcescens *is presented. It contains a transcription termination control site in the transcribed leader region preceding the first structural gene of the operon as well as a regulated promoter/operator transcription initiation region. The structural organisation of the leader region differs substantially from that of* Escherichia coli.

In *Escherichia coli*, transcription of the *trp* operon is regulated at a promoter/operator[1–4], and also at an attenuator[5,6]. The attenuator is a site within the transcribed leader region of the operon at which transcription is either terminated or allowed to proceed into the structural genes. Termination at this site seems to occur in response to the availability of charged $\mathrm{tRNA}^{\mathrm{Trp}}$ for protein synthesis[7,8]. RNA transcribed from the *trp* leader region contains a functional ribosome binding site[9–11], and may code for a short leader peptide with two adjacent tryptophan residues[12].

Comparison of the nucleotide sequences of the *trp* promoter/operator/leader region in *E. coli* and the related enterobacteria *Salmonella typhimurium* and *Shigella dysenteriae* have been useful in identifying the functionally important sequences within these regions[12–14]. The enterobacterium *Serratia marcescens* is more distantly related to *E. coli* and the sequence of its *trp* regulatory region would provide an interesting comparison. Although little is known of the structural organisation of the *trp* genes in *S. marcescens*, mutant and physiological studies indicate that many of these genes are arranged in an operon whose expression is coordinately regulated in response to changes in tryptophan concentration[15–17]. We have cloned a portion of the *S. marcescens trp* operon on plasmids and present here the nucleotide sequence of the regulatory region.

Cloning and restriction of *trp* operon

Chromosomal DNA of *S. marcescens* wild-type strain SM6 was cleaved with restriction endonuclease *Sal*I and the fragments inserted into the *Sal*I site of pBR322 (ref. 18). Plasmids carrying a functional *S. marcescens trpE* gene were isolated from the ligation reaction by selecting Trp^+ transformants of an *E. coli* recipient strain in which most of *trpE* is deleted (see legend to Fig. 1). Each of several Trp^+ transformants examined carried plasmids containing one or two inserts into the *Sal*I site of pBR322. One typical representative, pGM14, was chosen for further characterisation. It carries a single 3,000-base pair insert; a restriction map of this fragment is shown in the top portion of Fig. 1. Although pGM14 confers prototrophy to *trpE*$^-$ recipient strains, it does not complement lesions in any of the other *trp* structural genes, indicating that *trpE* is the only intact *S. marcescens trp* gene on pGM14.

S. marcescens trpE was located within the 3,000-base pair *Sal*I fragment by constructing derivatives of pGM14 carrying portions of the insert. One such plasmid, pGM63, carries only the right-hand half of the 3,000-base pair insert, from the *Sal*I site to the first *Eco*RI site (Fig. 1), but retains a functional *trpE*. This 1,500-base pair fragment could code for a protein of approximately 60,000 molecular weight, the estimated size[21–23] of *S. marcescens* anthranilate synthetase component I, the *trpE* product. This suggests that the *Eco*RI and *Sal*I sites delimiting the 1,500-base pair fragment must be very close to the ends of *trpE*. This was confirmed by DNA sequence analysis and comparison of the nucleotide sequences with the known N-terminal amino acid sequences of the *S. marcescens trpE* and *trpG* proteins[23]. The DNA sequence at the *Eco*RI end of the fragment correlates precisely with the amino acid sequence at the beginning of the *trpE* protein, with the *Eco*RI site preceding the AUG start codon by 20 base pairs. There are two back-to-back potential AUG translation start codons at the beginning of *trpE* (Fig. 2). Using the first, the N-terminal amino acid sequences of the *trpE* proteins of *S. marcescens*, *E. coli* and *Sal. typhimurium* align perfectly[23]. The *Sal*I end of the fragment is located about 30 base pairs beyond the start of *trpG*, the structural gene for anthranilate synthetase component II, which must therefore follow *trpE* in the *trp* operon of *S. marcescens*[16,26] (C.Y., G.F.M., G. Bennet and M. van Cleemput, in preparation). Thus, the 1,500-base pair *Eco*RI–*Sal*I fragment carries *trpE* intact. However, the 20 base pairs preceding the start of *trpE* are insufficient to function as a promoter and we must therefore assume that in plasmid pGM63 *trpE* is expressed from a plasmid promoter. Most of the regulatory region of the *S. marcescens trp* operon is therefore absent from plasmid pGM63, but is contained in the parent plasmid pGM14. A detailed restriction map of the DNA region immediately preceding the *Eco*RI site is shown in the bottom portion of Fig. 1.

DNA sequence of the promoter/operator/leader region

A DNA segment of approximately 380 base pairs around the *Eco*RI site at the beginning of *trpE* was sequenced using several overlapping restriction fragments (bottom portion of Fig. 1). Large portions of the DNA sequence of the entire $Eco\mathrm{RI}_{260}$ fragment and of the initial part of *trpE* (Fig. 2) are highly conserved between *S. marcescens*, *E. coli* and *Sal. typhimurium*[3,12,13]. This suggests that the overall organisation of the regulatory region of the *trp* operon is similar in the three organisms. In particular, a DNA segment centred around base pair −10 of the *S. marcescens trp* operon is almost perfectly conserved. In *E. coli* and *Sal. typhimurium* this region is essential for *trp* promoter and operator function[3,4,13,14]. Indeed, *E. coli* Trp repressor will protect the *Hpa*I site at positions −9 to −14 and the *Tac*I site (−20 to −23) but not the *Hin*cII site (−32 to −37) or the *Hae*III site (+15 to +18) of *S. marcescens* from cleavage by the respective restriction enzymes (data not shown). As in the case of *E. coli* and *Sal. typhimurium*, the *S. marcescens trp* operon contains a leader region which separates the promoter/operator region from the start of the first structural gene, *trpE*. In *E. coli* and *Sal. typhimurium* this region is transcribed[27] and contains the *trp* attenuator[6,12].

In vitro transcription of the initial portion of the *S. marcescens trp* operon

When DNA fragments containing the *trp* promoter/operator/leader region of *E. coli* or *Sal. typhimurium* are transcribed with purified RNA polymerase *in vitro*, a major RNA transcript is produced as a result of transcription termination at the *trp* attenuator[28]. To determine whether a similar termination site is

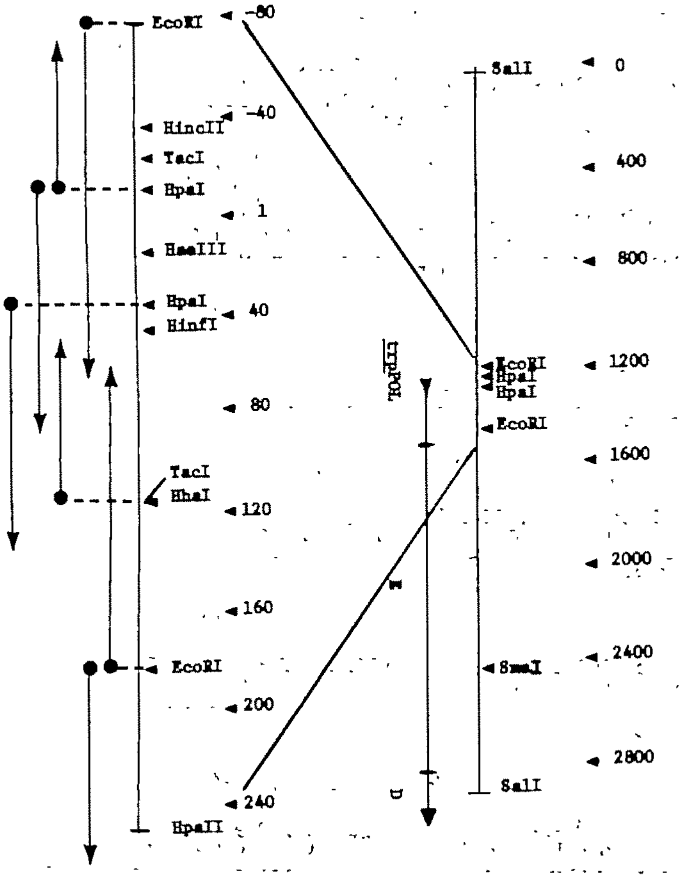

Fig. 1 Restriction map of a cloned fragment containing the initial portion of the *S. marcescens trp* operon. Chromosomal DNA of *S. marcescens* was prepared by the method of Miura and Sato[19]. 10 μg of chromosomal DNA and 5 μg of pBR322 DNA were digested with restriction endonuclease *Sal*I, phenol extracted, ethanol precipitated and resuspended in 250 μl of ligation buffer (60 mM Tris–HCl *p*H 7.6, 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP). The mixture was incubated in the presence of T4 DNA ligase for 2 h at 13 °C. Ligation was stopped by ethanol precipitation and the pellet resuspended in 100 μl TE-buffer (10 mM Tris–HCl *p*H 7.9, 0.1 mM EDTA). 20 μl were used to transform *E. coli* strain JA221 (C600 r^-m^+ Δ*trpE5 leu recA*) to prototrophy. About 20 ampicillin-resistant, tetracycline-sensitive Trp^+ transformants were obtained. As Trp^+ transformants were readily obtained using several restriction enzymes and two plasmid vehicles we believe that the cloned *S. marcescens* fragments contain unchanged chromosomal sequences. The transformation frequency (10 transformants per μg DNA) was comparable to that reported for *E. coli* DNA[20]. The restriction map of the insert carried by plasmid pGM14, a typical representative of the plasmids isolated from the Trp^+ transformants, is shown in the top portion of the figure. The arrow indicates the site of initiation and the direction of *trp* mRNA transcription. The bottom portion of the figure shows a detailed restriction map of the promoter/operator/leader region of the *S. marcescens trp* operon. Numbering starts at the presumed site of transcription initiation (position +1). The arrow indicates the direction and length of sequencing runs.

present in the leader region of the *S. marcescens trp* operon we isolated and transcribed various DNA fragments spanning the presumptive *trp* promoter/operator region. The transcripts obtained with the *Eco*RI_{260} and the *Hpa*II_{400} fragments are shown in Fig. 3. The *Eco*RI fragment ends at position +185 in the leader region whereas the *Hpa*II fragment extends to position +252 in the initial part of *trpE* (Fig. 1). Both fragments yield an approximately 175-nucleotide RNA transcript as the major product of *in vitro* transcription, together with a larger transcript of ~185 nucleotides for the *Eco*RI fragment and ~250 nucleotides for the *Hpa*II fragment. As the 175-nucleotide transcript is also observed when larger DNA fragments or plasmid pGM14 are used as DNA templates for *in vitro* transcription (results not shown), we conclude that the *S. marcescens trp* leader region contains a transcription termination site approximately 175 base pairs downstream from the transcription initiation site. Indeed, the DNA region between base pairs +150 and +180 shows close homology to the termination regions in the *E. coli* and *Sal. typhimurium trp* leader regions (Fig. 2)[12,28]. The larger transcripts observed with both the *Eco*RI_{260} and the *Hpa*II_{400} fragments (Fig. 3) presumably arise by readthrough beyond the transcription termination site to the end of the DNA fragments. Quantitative measurements indicate that there is about 30% readthrough at the *S. marcescens trp* leader termination site *in vitro*. In *E. coli* and *Sal. typhimurium* readthrough at the *trp* attenuator *in vitro* has been estimated at 5% and 30%, respectively[28]. In experiments in which we have examined the function of the *S. marcescens trp* attenuator in *E. coli*, tryptophan starvation relieved transcription termination and the increase in operon expression apparently exceeded that typical of the *E. coli trp* operon. Hutchinson and Belser[16] had previously noted that the regulatory response of *S. marcescens* to exogeneous tryptophan was five times more sensitive than that of *E. coli*.

Features of the promoter/operator region

Both DNA·RNA hybridisation data[29] and protein primary structure comparisons[23,30,31] indicate considerable evolutionary divergence in the *trp* operon of *S. marcescens* relative to those of *E. coli* and *Sal. typhimurium*. Conserved regions in such distantly related organisms may indicate functional importance. In particular, one might expect key sequences involved in recognition and binding of RNA polymerase and/or regulatory proteins to be conserved.

The promoter/operator regions of the *trp* operons of *E. coli* and *S. typhimurium* share a recognition sequence for *Hpa*I (GTT↓AAC) (Fig. 2). The integrity of this 6-base pair sequence seems to be essential for both operator and promoter function, as a single base pair alteration within this region severely affects both functions[14] (G. Zurawski, D. Elseviers and C.Y., unpublished). Of 19 base pairs in the symmetrical regions surrounding this *Hpa*I site, 18 are conserved between *E. coli*, *Sal. typhimurium* and *S. marcescens* (Fig. 2). *E. coli* Trp repressor recognises this DNA segment in all three organisms. In *S. marcescens* it protects two restriction sites—the *Hpa*I site between −9 and −14 and the *Tac*I site between −20 and −23—from cleavage by the respective enzyme. The only difference between *S. marcescens* and *E. coli* in this region, a CG instead of a TA base pair at position −16, does not seem to affect repressor–operator recognition significantly, although it does reduce symmetry. Interestingly, an *E. coli* mutant with a different base pair change at the same position (TA → GC) is O° (ref. 4), and Trp repressor does not protect its *Hpa*I site against cleavage by *Hpa*I (K. D. Brown and C. Y., unpublished results). The lack of sequence conservation from base pair −21 to −31 (Fig. 2) is consistent with the view that this DNA segment is not involved in repressor recognition[4]. The rightward endpoint of the region required for operator function in *E. coli* has been mapped to between positions −5 and +1 (ref. 4). The pattern of sequence conservation in this region (Fig. 2) suggests that residues to the right of position −3 may not be required for operator function. Taken together, these findings indicate that the *trp* operator of all three organisms is contained within the highly conserved DNA segment between residues −3 and −21.

RNA polymerase–promoter recognition and formation of a functional initiation complex are thought to involve sequence specific contacts with a DNA sequence around position −35 and with the 20-base pair DNA segment which immediately precedes the transcription initiation site[13,32]. The pattern of sequence conservation between *E. coli*, *Sal. typhimurium* and *S. marcescens* is consistent with this notion. In addition to the highly conserved sequence between positions −3 and −21 the three organisms have an identical 6-base pair sequence between positions −32 and −37 (Fig. 2). This sequence, GTTGAC,

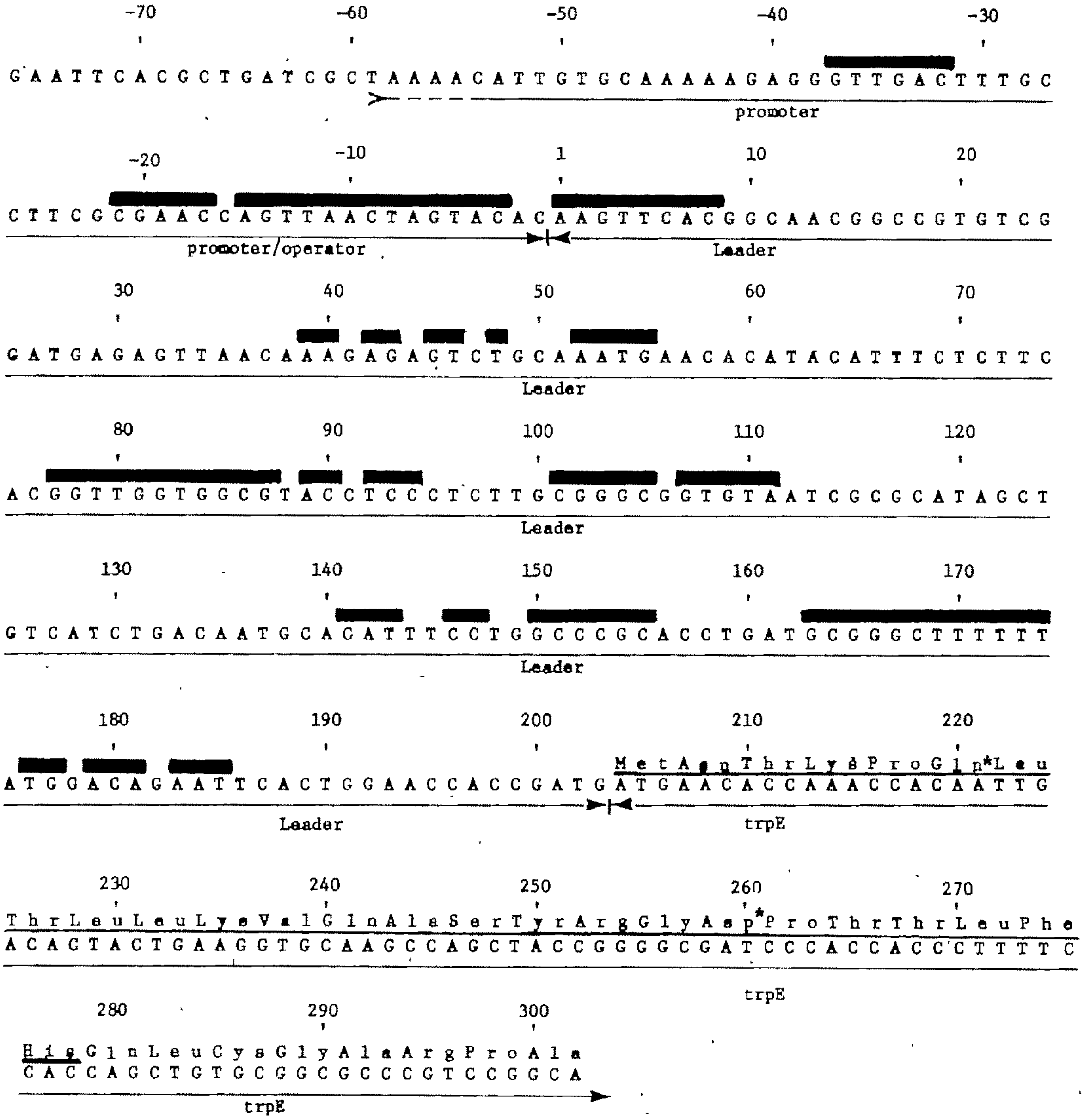
-70
-60
-50
-40
-30
GAATTCACGCTGATCGCTAAAACATTGTGCAAAAAGAGGGTTGACTTTGC
promoter
-20
-10
1
10
20
CTTCGCGAACCAGTTAACTAGTACACAAGTTCACGGCAACGGCCGTGTCG
promoter/operator
Leader
30
40
50
60
70
GATGAGAGTTAACAAAGAGAGTCTGCAAATGAACACATACATTTCTCTTC
Leader
80
90
100
110
120
ACGGTTGGTGGCGTACCTCCCTCTTGCGGGCGGTGTAATCGCGCATAGCT
Leader
130
140
150
160
170
GTCATCTGACAATGCACATTTCCTGGCCCGCACCTGATGCGGGCTTTTTT
Leader
180
190
200
210
220
MetAsnThrLysProGln*Leu
ATGGACAGAATTCACTGGAACCACCGATGATGAACACCAAACCACAATTG
Leader
trpE
230
240
250
260
270
ThrLeuLeuLysValGlnAlaSerTyrArgGlyAsp*ProThrThrLeuPhe
ACACTACTGAAGGTGCAAGCCAGCTACCGGGGCGATCCCACCACCCTTTTC
trpE
280
290
300
HisGlnLeuCysGlyAlaArgProAla
CACCAGCTGTGCGGCGCCCGTCCGGCA
trpE

Fig. 2 Nucleotide sequence of the regulatory region of the *S. marcescens trp* operon. The sequenced segment comprises the promoter/operator/leader region and the initial portion of *trpE*. This subdivision is indicated by the arrows. Numbering starts at the presumed site of transcription initiation (residue +1). Clusters of base pairs that have been conserved between *E. coli*, *Sal. typhimurium* and *S. marcescens* are indicated by black bars. With two exceptions (Gln instead of His at residue no. 6 and Asp instead of Asn at residue no. 19) the underlined portion of the predicted amino acid sequence of *trpE* is identical to the previously published amino acid sequence at the N-terminus of *S. marcescens* anthranilate synthetase component I (ref. 23). The two differences are indicated by an asterisk. The DNA sequence predicts a recognition site for restriction endonuclease *Hae*III (GGCC) between positions 149 and 152. We find no cleavage by *Hae*III at this site. As the *Hae*III recognition sequence partially overlaps an *Eco*RII recognition sequence (CCTGG) between positions 146 and 150, we assume that methylation of the complementary C residue at position 149 prevents recognition by *Hae*III. DNA sequence analysis was carried out by the method of Maxam and Gilbert[24]. Details of this and related procedures such as restriction site mapping, isolation of DNA fragments, phosphatase treatment, polynucleotide kinase reactions and agarose and polyacrylamide gel electrophoresis are given elsewhere[3,25].

recognised by *Hin*dII and *Hin*cII, occurs at the same relative position in several unrelated promoters[33]. The *S. marcescens trp* promoter is efficiently recognised by *E. coli* RNA polymerase both *in vivo* and *in vitro*. This suggests that the RNA polymerase of both organisms has similar requirements for its interaction with promoters. Initiation of transcription of the *S. marcescens trp* operon is likely to occur at the position previously determined for *E. coli*[27], particularly as 28 of 30 base pairs surrounding the potential initiation site (−21 to +9) are conserved. The importance of the highly conserved DNA segment between positions 1 and 8 following the transcription initiation site (Fig. 2) is unclear, as a deletion which replaces the DNA sequence beyond position +1 by a foreign sequence has no effect on either promoter or operator function in *E. coli*[3].

Leader region

A 200-base pair leader region separates the site of transcription initiation of the *S. marcescens trp* operon from the start of the first structural gene *trpE*. There are many functional and structural similarities between this DNA segment and the leader regions of the *E. coli* and *Sal. typhimurium trp* operons[12] and the *E. coli phe* operon[34]. They contain an attenuator region which terminates transcription in a run of TA base pairs preceded by a GC-rich segment. Although the residues at which transcription terminates have only been determined experimentally for the *E. coli* leader transcript[35,36], extensive sequence conservation in the region that contains the termination site in *E. coli* (Fig. 2; residues 140–180) suggests that in *S. marcescens* transcription termination must occur within the run of TA base pairs between residues 169 and 175. This is consistent with the estimated length of 175 nucleotides for the terminated *S. marcescens trp* leader transcript synthesised *in vitro*.

Like the other *trp* leader transcripts, that from *S. marcescens* could generate considerable secondary structure. The structure depicted in Fig. 4 is remarkably similar to those proposed for the *trp* and *phe* leader transcripts of *E. coli* and the *trp* leader transcript of *Sal. typhimurium*[28,34]. It has alternative stem and loop structures involving a GC-rich segment (residues 150–156) able to pair with two different regions of leader RNA. Base pairing with residues 162–168 generates the smaller stem and loop structure immediately preceding the 3′-OH end of the leader transcript. This structure has a calculated free energy of formation of $\Delta G \cong -17\ \mathrm{kcal\ mol^{-1}}$ (refs 37, 38). Studies of termination relief mutants suggest that the integrity of the analogous structure in the *E. coli trp* leader transcript may be essential for efficient transcription termination[39]. Residues 136–156 can also pair with residues 99–120 to form the alternative larger stem and loop structure, with a free energy of $\Delta G \cong -22\ \mathrm{kcal\ mol^{-1}}$. With the exception of the sequences corresponding to the unpaired loop regions (residues 121–135 and 157–161), the entire DNA segment responsible for the formation of the alternative stem and loop structures (residues 100–170) is highly conserved between *S.marcescens*, *E. coli* and *Sal. typhimurium* (Fig. 2).

The *trp* and *phe* leader regions examined have in phase translation start and stop codons and may thus code for a short leader peptide. In *E. coli*, and AUG-centred ribosome binding site early in the *trp* leader region[9] can function efficiently in initiation of translation both *in vivo* and *in vitro*[10,11]. Two tandem tryptophan codons are found in the sequence coding for the putative *trp* leader peptides of *E. coli* and *Sal. typhimurium*[12], whereas seven phenylanine residues are clustered in the C-terminal portion of the predicted *phe* leader peptide of *E. coli*[34]. Two potential AUG translation start codons are present in the early part of *S. marcescens trp* leader RNA (Fig. 4). Both are in phase with the tandem tryptophan codons (residues 80–85) and a UAA translation stop codon (residues 110–112). The number of amino acid residues predicted for the *S. marcescens trp* leader peptide is either 19 or 28, depending on which initiation site is used (Fig. 5). Although in the other *trp* and the *phe* leader transcripts the translation stop codon immediately precedes the region involved in the formation of the alternative stem and loop structures, in *S. marcescens* the UAA stop codon is five amino acid codons beyond this point, within the region of assumed secondary structure (Fig. 4). A highly conserved sequence surrounds the tandem tryptophan codons at residues 80–85 (Fig. 5). The only nucleotide differences between *S. marcescens* and either *E. coli* or *Sal. typhimurium* in this region, U instead of C at position 88 and C in place of U at position 91, do not affect the predicted amino acid sequence. This conserved sequence is in the same position relative to the large stem and loop structure in all three *trp* operons. Although we believe that the extent of translation of the two tryptophan codons is an integral feature of the mechanism governing regulation of transcription termination[40], we cannot explain the extensive nucleotide and amino acid sequence conservation of the region surrounding the tryptophan codons.

Comparison of the initial portion of the *S. marcescens trp* leader region with the analogous regions in both *E. coli* and *Sal. typhimurium* reveals that the *S. marcescens trp* leader region contains a DNA insert of 27 base pairs between residues 9 and 10 of the *E. coli* and *Sal. typhimurium* sequences (Fig. 4). The 7 base pairs preceding this DNA segment (AGTTCAC; residues 2–8) are repeated with one difference at the end of the insert (AGTTAAC; residues 31–37). This extra DNA codes for the first of the two potential translation start codons (residues 26–28) for the *S. marcescens trp* leader peptide. The position of this AUG codon relative to the transcription initiation site is similar to that of the initiator codons for the *E. coli* and *Sal. typhimurium* leader peptides. The second potential start codon for the *S. marcescens trp* leader peptide is between residues 53 and 55 (Fig. 5). The region preceding and including this AUG codon has been moderately conserved (Fig. 2). The region preceding either potential start site for the *S. marcescens trp* leader peptide shows no obvious homology to known ribosome binding sites or to the 3′ end of 16S rRNA[41].

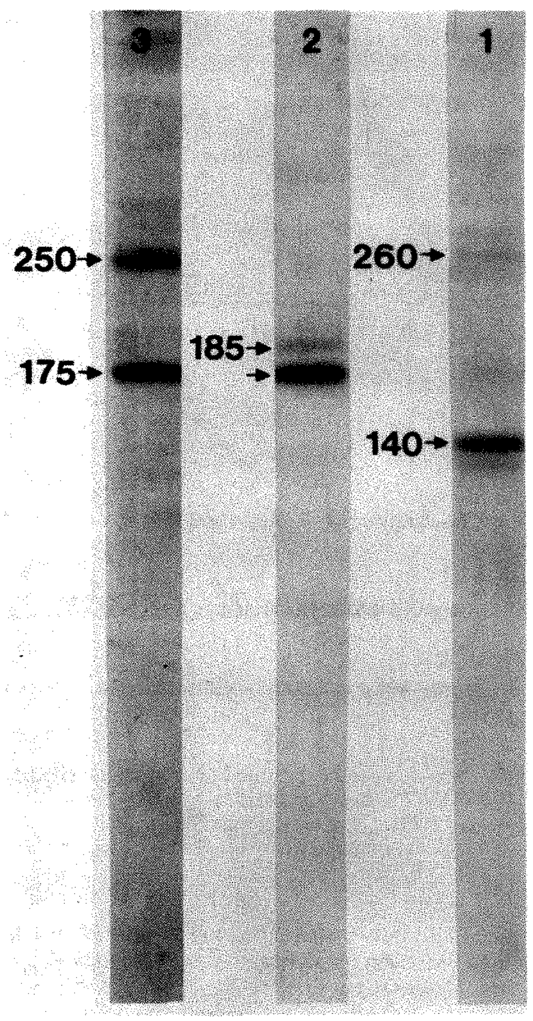

Fig. 3 *In vitro* transcription of DNA fragments containing *S. marcescens trpPOL*. RNA was transcribed from the following DNA fragments: (3) *S. marcescens Hpa*II$_{400}$, (1) *S. marcescens Eco*RI$_{260}$, (1) *E. coli Hpa*II$_{570}$. All fragments contain the regulatory region of the respective *trp* operons. The arrowed numbers indicate the length in nucleotides of the various transcripts. The size of the *S. marcescens* transcripts (slots 3 and 2) was calculated from the known lengths of the transcripts obtained with the *E. coli Hpa*II$_{570}$ fragment[28]. The 175-base RNA bands arise by transcription termination at the *S. marcescens trp* attenuator; the 185- and 250-base RNA bands are the result of transcription beyond the transcription termination site to the end of the respective fragments. The composition of the *in vitro* transcription mixture as well as the separation of the transcription products on polyacrylamide gels has been described[28].

We do not know whether either or both of the potential start sites is used in *S. marcescens*. Lee and C.Y.[28] have proposed a model for the regulation of transcription termination that is based on translation of the leader transcript and a shift between alternative RNA base pairing configurations in this transcript. Recent evidence suggests that base pairing between residues in the large loop of the alternative stem and loop structures and proximal segments of RNA may also be involved in attenuation. Thus, a stable hydrogen-bonded structure involving the large

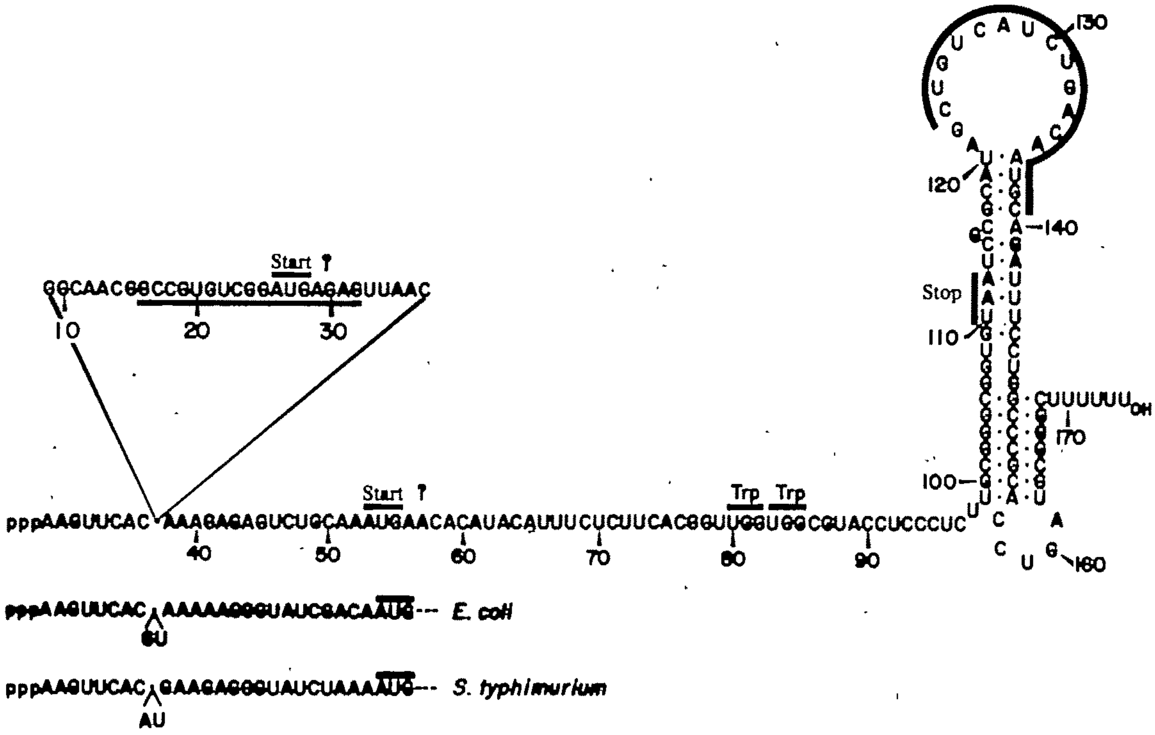
Start
GGCAACGGCCGUGUCGGAUGAGAGUUAAC
10
20
30
Start
pppAAGUUCAC AAAGAGAGUCUGCAAAUGAACACAUACAUUUCUCUUCACGGUUGGUGGCGUACCUCCCUC
40
50
60
70
80
90
Trp Trp
100
110
Stop
120
130
140
160
170
CUUUUUU
OH
pppAAGUUCAC AAAAAGGGUAUCGACAAUG--- E. coli
GU
pppAAGUUCAC GAAGAGGGUAUCUAAAAUG--- S. typhimurium
AU

Fig. 4 Proposed secondary structure of the terminated *S. marcescens trp* leader transcript. The left hand portion of the diagram shows the position of the 27-base pair 'insert' compared to the sequence of the initial portion of the *E. coli* and *Sal. typhimurium trp* leader transcripts. The locations of the two possible translation start codons, the tandem tryptophan codons and the translation stop codon, are indicated. The regions of sequence homology between the insert and the large loop of the alternative secondary structures are indicated by underlining.

Loop of the alternative stem and loop structures and the region containing the seven phenylalanine codons can be drawn for the *phe* operon transcript of *E. coli*[34]. A similar, though weaker, complementarity between the region containing the two tryptophan codons and some of the residues of the large stem and loop exists in the *E. coli* and *Sal. tyhimurium trp* leader transcripts[40]. In *S. marcescens*, the complementarity (between residues 89–95 and 102–108) is restricted to the RNA region distal to the two tryptophan codons. However, a unique feature of the *S. marcescens trp* leader RNA is the strong complementarity between the region of the transcript which forms the large loop and the RNA sequence corresponding to the DNA insert (Fig. 4). Of 17 nucleotides between positions 16 and 32, 14, including the first AUG codon, are complementary to residues 139–123 of the *S. marcescens trp* leader transcript. The calculated free energy of formation of this structure is about -10 kcal mol^{-1}.

Although many aspects of the functional and structural organisation are similar in all *trp* leader regions examined so far, three obvious differences exist between the *trp* leader region of *S. marcescens* and the analogous regions of *E. coli* and *Sal. typhimurium*: (1) an extra segment of DNA, 27 base pairs long, inserted into the early portion of the *S. marcescens trp* leader region; (2) potential base-pairing between nucleotides within this insert and residues in the large loop of the alternative stem and loop structures; (3) the potential of *S. marcescens trp* leader RNA to code for a 19- or 28-residue leader peptide, with a stop codon within the region assumed to be involved in the formation of an alternative stem and loop structure. Despite these differences, the *S. marcescens trp* leader region terminates transcription both *in vitro* and *in vivo*, like the analogous regions in *E. coli* and *Sal. typhimurium*, and the extent of termination *in vivo* is a function of the availability of tryptophan. Our results suggest that regulation of transcription termination at the *trp* attenuator of *S. marcescens* may allow a greater range of expression than is attainable at the *E. coli trp* attenuator. The structural differences between the leader regions of *E. coli*, *Sal. typhimurium* and *S. marcescens* may therefore have developed to allow quantitatively different regulatory responses.

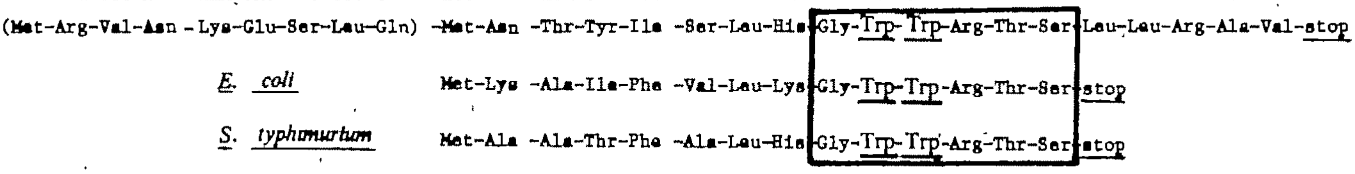
30
40
50
60
70
80
90
100
110
AUG AGA GUU AAC AAA GAG AGU CUG CAA AUG AAC ACA UAC AUU UCU CUU CAC GGU UGG UGG CGU ACC UCC CUC UUG CGG GCG GUG UAA
(Met-Arg-Val-Asn -Lys-Glu-Ser-Leu-Gln) -Met-Asn -Thr-Tyr-Ile -Ser-Leu-His-Gly-Trp-Trp-Arg-Thr-Ser-Leu-Leu-Arg-Ala-Val-stop
E. coli
Met-Lys -Ala-Ile-Phe -Val-Leu-Lys-Gly-Trp-Trp-Arg-Thr-Ser-stop
S. typhimurium
Met-Ala -Ala-Thr-Phe -Ala-Leu-His-Gly-Trp-Trp-Arg-Thr-Ser-stop

Fig. 5 Predicted amino acid sequences of *trp* leader peptides. The predicted amino acid sequence of the putative *S. marcescens trp* leader peptide (second line) is compared with the predicted sequence of the analogous peptides of *E. coli* and *Sal. typhimurium*. The three peptides share an identical sequence of six amino acids surrounding the tandem tryptophan codons (indicated by a box). The *S. marcescens trp* leader peptide extends five amino acid residues beyond the end of the other peptides at its C-terminus. Depending on which of the two potential translation initiation sites is used, it may start at the same position as the *E. coli* and *Sal. typhimurium* peptides, or it may have nine additional amino acid residues at its N-terminus (indicated in parentheses).

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The regulatory region of the biotin operon in *Escherichia coli*

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It is proposed that the biotin anabolic operon in Escherichia coli *is transcribed divergently from two partially overlapping face-to-face promoters. A mutation that increases transcription* in vivo *creates an additional promoter* in vitro. *The putative operator contains an imperfect palindromic sequence that partially overlaps the promoters. The regulatory and genetic implications of these findings are discussed.*

THE synthesis of biotin in *E. coli* requires the products of the *bio* gene cluster[1,2], which is located at approximately 17 min on the genetic linkage map[3]. Transcription of the *bio* gene cluster is divergent[4,5]. Transcription is initiated between the *bioA* and *bioB* genes and proceeds to the left into *bioA* and, from the opposite DNA strand, to the right into *bioB*, *F*, *C*, and *D*[4,5]. Transcription in both directions is co-repressed by biotin[4,6,7]. Genetic studies by Ketner and Campbell[8,9] have shown that the *bioA* and *bioB* promoters are not independent, as several single mutations which increase *bioA* transcription decrease *bioB* transcription. The operators are not independent; a single mutation results in constitutive RNA synthesis in both directions[9]. In this report, we propose that the *bioA* and *bioB* promoters are situated face-to-face and partially overlap. The operator contains a, hyphenated palindromic sequence that partially overlaps the promoters. This model is similar to one of the three models proposed by Ketner and Campbell[9].

We have evidence that one of the mutations[9] that increases leftward transcription, *bioP*98, may create a second leftward promoter. In addition, we report the nucleotide sequence of the *bio* regulatory region.

Identification and isolation of the *bio* regulatory region

Among the *bio* regulatory mutants isolated by Ketner and Campbell[9] were promoter down mutants that were unable to transcribe in either direction (Table I). The promoter down mutation, *bioP*132, was recovered by transduction with λ*bio*1 and the resultant phage, λ*bio*132, was found to contain a DNA insertion element[9], presumably within the *bio* regulatory region. (To simplify identification of bacteriophage λ*bio*1 derivatives, a shorthand description will be given to each transducing phage. This numbering[9] does not infer isolation of an independent transducing phage with endpoints of the *bio* substitution different from λ*bio*1. The phages described in this article are as follows: λ*bio*132 is λ*bio*1*bioP*98*bioP*132 and λ*bio*234 is λ*bio*1*bioP*98*bioO*34. The DNA insertion, which seems to be IS*1* from restriction site data, serves as a convenient physical marker for detecting restriction fragments that contain the *bio* regulatory region. Various restriction digests[10] of λ*bio*1 and λ*bio*132 DNA were compared by gel electrophoresis[11,12]. The *Hin*dII sites in the *bio* substitutions of these phages are shown in Fig. 1. Further analysis resulted in the partial restriction map in Fig. 2.

It was necessary to locate the *bio* control region by another method as it is possible that the IS element could inactivate transcription by a mechanism unrelated to its insertion. Proof that fragments containing the site of the DNA insertion also contain the *bio* regulatory region came from the comparison of *in vitro* transcription of wild type (λ*bio*1) and mutant (λ*bio*234) DNA fragments. Transcription of the *Hin*dII fragment from λ*bio*1 that was postulated to contain the control region (from the position of IS*1* in an analogous fragment from λ*bio*132) gave a major transcript of 170 nucleotides. This transcript terminated at the *Hin*dII site and hybridised to the l-strand of *bio* transducing phage DNA, identifying it as a leftward (*bioA*) transcript.

On the other hand, a DNA fragment from λ*bio*234, which contains the promoter up mutation, *bioP*98, and the operator constitutive mutation, *bioO*34, gave two major transcripts, one of 170 nucleotides and another of 150 nucleotides, both hybridising to the *l*-strand of *bio* transducing phage DNA. The longer transcript was initiated at the wild-type start site and the shorter transcript was initiated 24 nucleotides down from the wild-type

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Table 1 Three classes of *bio* regulatory mutants

	Presence of *galT* and *E*	Phenotype Low biotin	Phenotype High biotin	Properties
Parent (*bio*Δ61)	+	Gal$^-$	Gal$^-$	Gal$^-$ at low biotin due to insufficient transcription.
	−	Gal^S	Gal^R	
I Promoter A up mutants P98 (spontaneous) P2, P10, P43 (2AP)	+	→Gal$^+$	Gal$^-$	*bioA* mRNA increased 4- to 6-fold, *bioB* mRNA decreased to one-third wild type.
	−	Gal^S	ND	
II Promoter A down mutants P130, P131, P132 (2AP)	+	Gal$^-$	Gal$^-$	No transcription in either direction, IS element in regulatory region.
	−	→Gal^R	Gal^R	
III Operator constitutive mutants O34, O38, O49, O99 (spontaneous)	+	Gal$^+$	→Gal$^+$	Constitutive in both directions, same amount of *bioA* mRNA as *bioP*98, *bioB* mRNA increased twofold.
	−	Gal^S	Gal^S	

The data in this table are summarised from ref. 9. The *bio* operons are under negative control with biotin as the co-repressor. Low levels of biotin (1.6 nM) permit synthesis of biotin, while high levels of biotin (41 nM) repress synthesis. Both a positive and negative selection technique were used to isolate control mutants. The parent strain contains a deletion (*bio*Δ61) which fuses *bioA* to *galE* and places the galactose operon under the control of the *bio* regulatory system. A portion of both *bioA* and *galE* are deleted in this strain. If *galK* is expressed, for example at low biotin concentrations, in the presence of galactose (glycerol is the carbon source) the accumulation of galactose-*1*-phosphate prevents growth. This is a negative selection for galactose utilisation. If, on the other hand, *galE* (and *galT*) is supplied by a transducing phage integrated at a distant site, galactose is metabolised and can serve as the sole carbon source. This is a positive selection for galactose utilisation. By using these two selections, Ketner and Campbell[9] isolated the three classes of regulatory mutants listed. In each case, an arrow designates the ancestry of each of the mutant types. Thus the second and third classes of mutants all contain the *bioP*98 mutation. Abbreviations are as follows: Gal$^-$, unable to utilise galactose; Gal$^+$, able to utilise galactose; Gal^S, sensitive to galactose; Gal^R, resistant to galactose; 2AP, induced with 2-aminopurine; ND, no data.

start site. These results correlate *in vitro* and *in vivo* transcriptional changes. If the shorter transcript were due to *bioP*98 rather than *bioO*34, it would explain the total fourfold increase in leftward transcription in strains containing *bioP*98 which does not change further on the formation of the double mutant, *bioP*98, *bioO*34. The *bioP*98 mutation may increase transcription by creating a second leftward promoter, *bioP*98, whilst maintaining the wild-type *bioA* promoter. A precedent for overlapping promoters has been established in the galactose operon of *E. coli*[13].

Early transcription experiments were carried out in the presence of heparin to produce selective transcription[14]. In these conditions, very little *bioB* transcription occurs. Later experiments were carried out in the absence of heparin, and transcription was initiated by the addition of magnesium chloride[15]. Selective transcription was maintained by using higher salt concentrations (see legend to Fig. 2). In this case, rightward transcription is increased to about one-half the level of leftward transcription.

Although we do not have direct genetic evidence for the positions of the *bioA* and *bioB* promoters, we have investigated the transcription of the *Hin*fI fragment and the *Hae*II fragment that contain the control region (Fig. 2). These two fragments together encompass a region 340 nucleotides to the left and 500 nucleotides to the right of the *bioP*98 promoter. In magnesium start conditions, two major transcripts could be found in each case; one leftward and one rightward. Transcripts from different fragments were shown to have the same start sites. Individual transcripts from the *Hin*fI fragment were hybridised to the separated strands of the *Hha*I fragment containing the control region and the unhybridised portions of the RNA removed with RNase[16]. Fingerprints[17] of the RNase-protected transcripts were essentially identical to those from transcripts from the *Hha*I fragment itself. Thus there are apparently only two major promoters in the 650-base pair *Hin*fI fragment.

Organisation of the regulatory elements

Both DNA strands of the *bio* control region have been sequenced. The sequence between the *Fnu*EI and *Hph*I sites is presented in Fig. 3. There are several prominent features of the control sequences. First, there is a large imperfect palindrome near the *in vitro* mRNA start sites. The promoters are situated face-to-face and partially overlap. Both promoters overlap the palindrome, but the *bioB* promoter does so to a much greater extent. The promoter sites are A-T rich (46/65 base pairs) and are flanked by relatively G-C rich sequences (16/25 and 9/19 base pairs). The first AUG codons of the two mRNAs are 86 nucleotides apart on the DNA sequence. It is not yet known whether these represent the initiation sites for the synthesis of the *bioA* and *bioB* gene products.

Positions of the promoters

There are three possible positions for the two *bio* promoters: (1) overlapping; (2) face-to-face and non-overlapping; (3) back-to-back and non-overlapping[9]. The first model can be divided into a spectrum of models that range from overlapping face-to-face to overlapping back-to-back promoters. The proposed promoters are found to conform to the first model with face-to-face

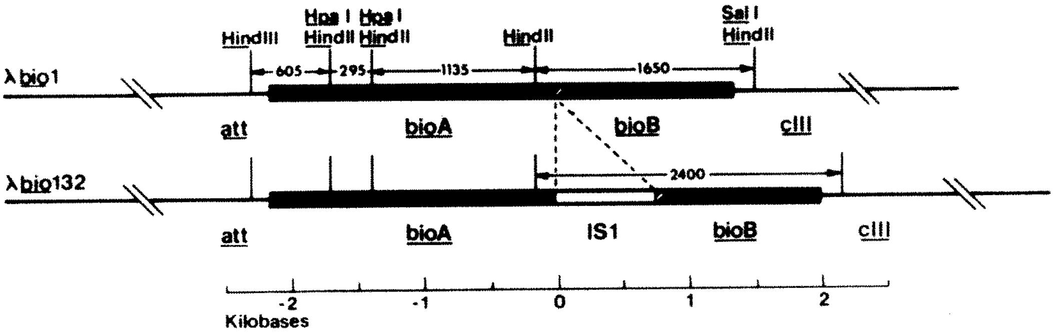


Fig. 1 The position of IS*1* (open line) within the *bio* substitution (thick line) in the λ*bio*132 genome locates the *bio* regulatory region (shaded line). The *Hin*dII and *Hin*dIII sites within and adjacent to the *bio* substitution are shown. The endpoints of the *bio* substitution in λ*bio*1 and λ*bio*132 are within 480 base pairs of the *Hin*dIII site and 530 base pairs of the *Sal*I site. The IS*1* elements in λ*bio*132 and in λ*dgalT*:S188 (ref. 20) are in the same orientation relative to the λ genome. The orientation of IS*1* is then '+' with respect to the *E. coli* genome[50]. Fragment sizes are in base pairs. The centre of the large 'palindrome' in the regulatory region is the zero point of the scale.

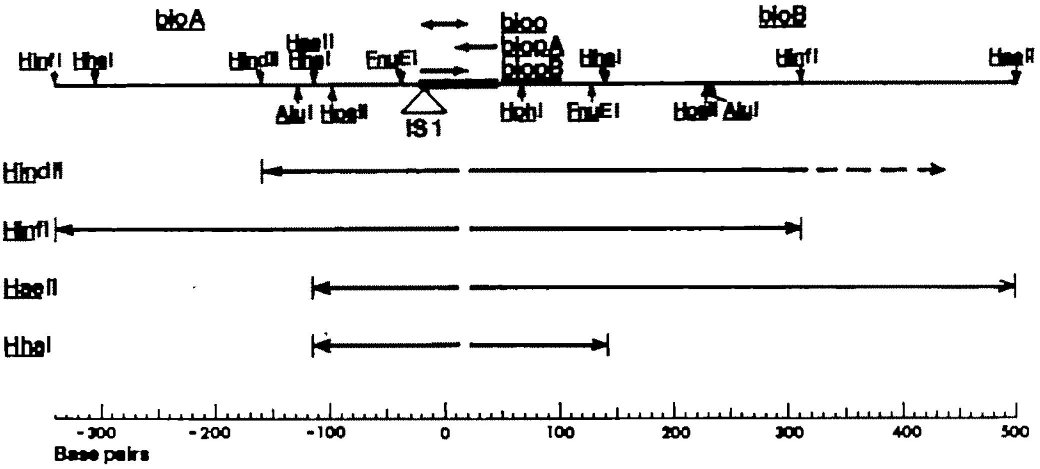


Fig. 2 The first restriction sites flanking the *bio* regulatory region are shown for several sequence-specific endonucleases. The thick line represents the region containing the *bioA* and *bioB* promoters. The positions of the promoters (*bioPA* and *bioPB*) and the operator (*bioO*) are shown by arrows above the linear map (see Fig. 3). The approximate position of the IS*1* insertion in *bioP*132 is shown. The lower portion of the figure shows the transcripts, (arrows), obtained from each of the wild-type restriction fragments listed at the left Only two initiation sites were observed in the entire 840 nucleotide region that is presented. The rightward transcript from the 1,650-base pair *Hin*dII fragment, represented as a broken line, terminates prematurely at several sites. Transcription experiments were carried out in a final volume of 20 μl with approximately 0.05–0.2 μg of a purified DNA fragment (about 10 nM) in 40 mM Tris-HCl at *p*H 8.0, 100 mM KCl and 10 mM 2-mercaptoethanol. RNA polymerase holoenzyme, 8.6 μg (840 nM or an RNA polymerase-to-DNA ratio of 84:1), was added to the reaction mix and incubated at 37 °C for 5 min. The reaction mixture was then transferred to a tube containing a single [α-^{32}P] ribonucleoside triphosphate at a final concentration of 10 μM for pyrimidine nucleoside triphosphates and 25 μM for purine nucleoside triophosphates, as well as the remaining required ribonucleoside triphosphates at final concentrations of 200 μM. The mixture was then incubated for 5 min at 37 °C and the reaction started by adding $MgCl_2$ to a final concentration of 10 mM. After 20 min of RNA synthesis, the reaction was worked up as described previously[14].

partially overlapping promoters. The *in vitro bioA* and *bioB* mRNA start sites are 10 nucleotides apart on the DNA and the messengers do not overlap. This observation rules out the second model, of two distant face-to-face promoters with overlapping transcripts, which was not ruled out genetically. The third model of two independent and distant back-to-back promoters has been proposed by Das Gupta *et al.*[18] on the basis of electronmicrographic evidence, but it is not supported by the promoter interactions found in the genetic studies[9]. However, the genetic argument requires that the promoters are functionally independent, which is not substantiated in the singular case of the interactive distant back-to-back promoters of the arabinose operons of *E. coli*[19]. Das Gupta and his collaborators believe that the mRNA start sites are about 174 nucleotides apart[18] but we have not found transcripts separated by this distance in our studies. We must assert, however, that only the position of the *bioP*98 promoter can be uniquely and unambiguously determined by mutation. The *bioA* and *bioB* promoters are positioned solely by the fact that *in vitro* transcription starts at two positions within an 840-base pair region that contains portions of the *bioA* and *bioB* genes.

The proposed orientation of the promoters is consistent with the genetic data. The *bioP*98 mutation, which increases total leftward transcription fourfold, decreases rightward transcription to one-third of the wild-type level[9]. Because the promoters overlap, mutations in one promoter may affect the other promoters. It is also possible that operator mutants affect promotion, and promoter mutants may affect repression, as the putative operator partially overlaps the promoters. The binding of RNA polymerase to the *bioA* or *bioP*98 promoters could even sterically prevent binding of RNA polymerase to the *bioB* promoter.

A second genetic observation that is explained by the proposed model has been noted by Ketner and Campbell[9]. The insertion sequence element found in the *bio* regulatory region could inactivate rightward transcription by insertion into the *bioB* promoter and could block transcription from the *bioA* promoter by termination within the IS*1* element. IS*1* is known to give polar mutations when inserted in either direction[20]. In this regard, we have mapped the insertion site to a region near or in the *bioB* promoter and to the left of the *bioA in vitro* mRNA start site (Fig. 2).

Finally, the proposed promoter orientation may explain an observation of Das Gupta *et al.*[18]. They observed transcriptional intermediates using the electron microscope in which one DNA strand had been displaced by an RNA strand synthesised in either direction, but not in both directions. For those intermediates observed, the binding of a second RNA polymerase molecule is precluded because the second promoter is in the form of an RNA–DNA hybrid.

Location of the operator

The selection of operator mutants that are simultaneously constitutive for transcription in both directions suggests that the promoters for both operons must share at least one common operator site[9]. However, studies of the repression of the *bio* operons with biotin analogues suggest the possibility of two independent operators[21]. Preliminary sequence analysis of the double mutant, *bioP*98, *bioO*34, shows two sequence changes. One of these is at position −3 (Fig. 3) and the other is at position +15. They are both located within the imperfect palindrome. We conclude that the imperfect palindrome is at least one component of the operator, but as we have sequenced neither mutation alone we cannot assign each mutation an identity. Because the *bio* repressor has not been isolated, it is not possible to locate the operator by binding studies; however, it is possible to select plasmids containing the *bio* operator by their ability to titrate repressor (A. O. and J. A., unpublished). The *bio* repressor(s), like the *gal*, *trp*, *lac* and λ phage repressors (reviewed in ref. 22), may effect repression by isosterically preventing RNA polymerase binding rather than by allosteric interaction.

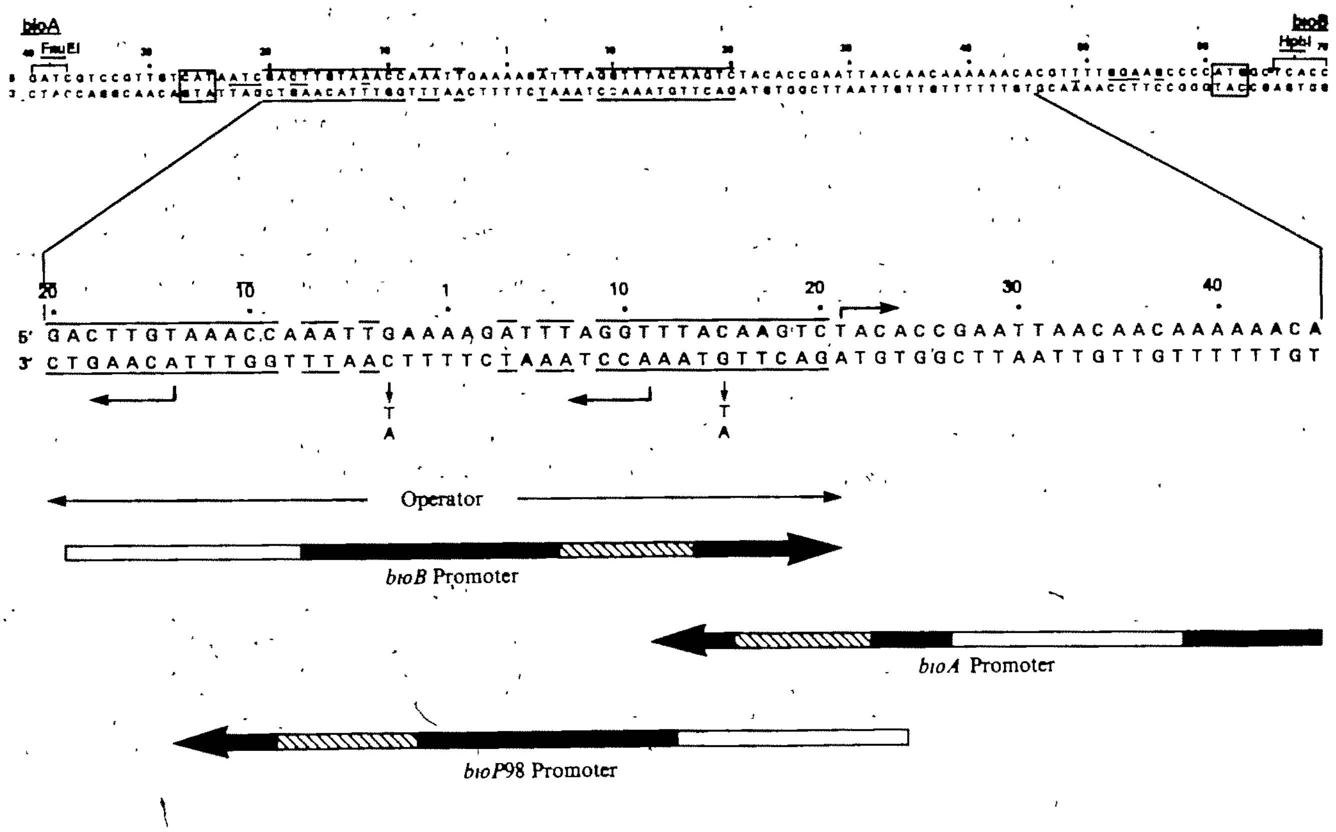
Promoter sequences

It is difficult to define the exact molecular interactions between RNA polymerase and a given promoter. The physical limits of promoters have been approximately defined by mutations and binding or protection studies[13,23–43,49]. However, most sequenced promoters have two common interaction sites. These are the Pribnow box[25] and the recognition site[27], both defined by homologies found in sequenced promoters, as well as, to a certain extent, by mutations or protection studies (reviewed in refs 23,24). The Pribnow box is of the form 5'TATRRTR3' (R, a purine nucleotide) and is centred at approximately 10 nucleotides proximal to the RNA initiation site. The A in the second position and the T in the sixth position from the 5' end of the Pribnow box are the most highly conserved bases[13]. In the *bioA* and *bioB* promoters four out of seven bases match the Pribnow paradigm (Fig. 4). There is an A residue in the second position and a T residue in the sixth position of the Pribnow box in each promoter. In the case of the *bioP98* promoter, four out of seven bases agree with the Pribnow sequence. The tentative C to A substitution in the *bioP98* promoter allows agreement with the second nucleotide of the formal Pribnow sequence. Because both the sequenced operator and promoter mutations occur within the *bioP98* promoter, the importance of the substitution at position −3 awaits sequencing of the *bioP98* mutation alone.

The second common feature of most promoters is a recognition site which encompasses about 12 nucleotides centred at approximately position 32 upstream of the RNA initiation site. There are two general types of recognition sites. However, there are some promoters that do not fit into either class very well, for example the *lacI* promoter[42], and others that are between classes, for example the *araB* promoter[43]. Nevertheless, it is convenient to divide the promoters into groups with recognition sequences most closely resembling the sequences TGTTGACAATTT, referred to here as type I promoters[23–35], and those resembling the sequence NNNNNACACTTT (N is any nucleotide), referred to here as type II promoters[13,23,36–41]. The former type is quite often found in bacteriophage promoters[25–33] and the latter in the catabolite activator protein (CAP) regulated sugar utilisation operons[13,23,36–40] in *E. coli*. The *bioA* promoter contains a recognition site that agrees with 9 of the 12 bases in the type I recognition site. However, there are only 5 bases between the *bioA* recognition site and the Pribnow box, in contrast to a distance of 12 to 14 bases for most promoters. Perhaps this distance can be reconciled by the possibility that two different subunits of RNA polymerase act at the two sites. Alternatively, the true recognition site may reside further from the Pribnow box and contain less homology to known recognition sites. The recognition site of the *bioB* promoter matches the type I sequence in five positions. This is not an unusually poor fit as several type I promoters agree with the type I sequence at only six positions[27,33]. The *bioP98* promoter fits at seven positions in the type I recognition sequence. The average match for the type I promoters evaluated here[25–35] is 8 out of 12 positions.

It is difficult to explain the relationship of the *in vivo* transcriptional levels to the primary sequence of the promoters. The *bioA* promoter produces one-twentieth of the molar amount of mRNA, when fully de-repressed in a Bio$^-$ strain, compared to the fully induced *galE* promoter[9]. The *bioA* and *bioB* promoters transcribe at approximately equal efficiencies *in vivo*[4]. The *bioP98* promoter is three times more efficient *in vivo* than the wild-type *bioA* promoter[9]. However, there is no obvious correlation between these promoter sequences and the resulting transcriptional levels. The *bioA* and *bioB* promoter sequences are quite different, yet they produce RNA equally well. It is not clear what features cause these promoters to be twenty times less efficient than the *galE* promoter. Lastly, because the *bioP98* and

Fig. 3 The overlapping face-to-face *bioA* and *bioB* promoters are positioned on the DNA sequence by the 5' ends of the *in vitro* mRNAs as well as homology to other promoters[23–43]. The *bioP98* promoter, created by mutation, is positioned by the 5' end of the mRNA as well as the tentatively assigned base substitutions at positions −3 and +15 found in the double mutant, *bioP98*, *bioO34*. The promoters are defined to be DNA sequences starting from and extending 35 to 40 nucleotides upstream from the initiation site for transcription. This definition is based on studies of DNA regions essential to promoter activity[23]. The *bioB* promoter and the *bioP98* promoter overlap the putative operator to a greater extent than the *bioA* promoter (lines above and below the DNA sequence delineate the imperfect palindrome). Transcription may be repressed sterically either by binding of repressor to the operator or by steric competition of RNA polymerase molecules for one of the several overlapping promoters. Neither the presumptive sequence change in the *bioO34* mutation, which abolishes repression *in vivo*, nor the presumptive *bioP98* sequence change, which increases total leftward transcription fourfold, affects sequences in the recognition sites (open lines) or Pribnow box structures[23] (shaded lines) of the *bioA* or *bioB* promoters. Both strands of the DNA have been sequenced from positions −21 to 53 by the method of Maxam and Gilbert[51]. RNA sequence data from the *bio* transcripts will be presented elsewhere. Sequences that are complementary to the 3' end of 16S rRNA[52] are underlined and the first AUG sequences in each RNA are enclosed in boxes.


bioA
bioB
FnuEI
HphI
20
10
1
10
20
30
40
5' GACTTGTAAACCAAATTGAAAAGATTTAGGTTTACAAGTCTACACCGAATTAACAACAAAAAAACA
3' CTGAACATTTGGTTTAACTTTTCTAAATCCAAATGTTCAGATGTGGCTTAATTGTTGTTTTTTTGT
T
A
T
A
Operator
bioB Promoter
bioA Promoter
bioP98 Promoter

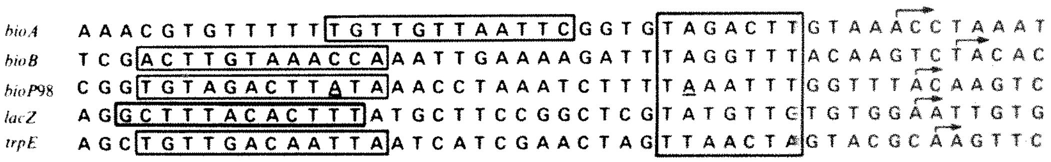

bioA A A A C G T G T T T T T T G T T G T T A A T T C G G T G T A G A C T T G T A A A C C T A A A T
bioB T C G A C T T G T A A A C C A A A T T G A A A A G A T T T A G G T T T A C A A G T C T A C A C
bioP98 C G G T G T A G A C T T A T A A A C C T A A A T C T T T T A A A T T T G G T T T A C A A G T C
lacZ A G G C T T T A C A C T T T A T G C T T C C G G C T C G T A T G T T G T G T G G A A T T G T G
trpE A G C T G T T G A C A A T T A A T C A T C G A A C T A G T T A A C T A G T A C G C A A G T T C

Fig. 4 The *bio* promoters are compared to the tryptophan[35] and lactose[36] operon promoters of *E. coli*. All promoters are aligned by their Pribnow boxes. The wild-type *bio* promoters are dissimilar in sequence, though the *bioP98* and *bioB* promoters share extensive homology due to the imperfect palindrome. All the *bio* promoters are more closely related to the tryptophan operon or bacteriophage promoters than to the lactose operon promoter. Sequences that are similar to the recognition site of the *trpE* promoter are enclosed in boxes. In the *bioA* promoter, these 'recognition' sequences are unusually close to the Pribnow box. The base substitutions in the *bioP98* promoter are underlined. The mRNA starts (arrows) were determined by an indirect method[53]. RNA samples were divided into two aliquots and one aliquot was treated with bacterial alkaline phosphatase to remove the triphosphate at the 5′ end. Comparison of RNase Tl fingerprints of the two samples immediately identified the 5′-oligonucleotide. We cannot yet rule out initiations of leftward transcription at positions 8, 9 or 10 or of rightward transcription at position 20. It is unusual that the rightward transcript is initiated with uridine-5′-triphosphate.

bioB promoters overlap the 'palindrome,' they share considerable homology. However, these promoters are not equally utilised *in vivo*.

Operator sequences

The putative *bio* operator, like the *lac*[44], *trp*[35], *gal*[40] and phage λ (ref. 30) operators, is an imperfect palindromic sequence and suggests a symmetrical repressor interaction. The imperfect palindrome is larger than any other sequenced operator and bears no sequence resemblance to any of the other operators. The palindrome could be increased by deletion of the A-T base pair at position 8 or an insertion of an A-T base pair between positions −8 and −9.

Comparison with other control regions

Recently, considerable progress has been made in determining the DNA sequences of several regulatory regions[23–43]. Comparative studies of the overall organisation of control regions as well as the individual regulatory elements have become possible. The *bio* system incorporates some of the general features of the unidirectional operons. Like *gal*, *lac*, *trp* and λp_L, the 'palindromic' operator overlaps the promoters. In the *bio* system we find an unusual transcriptional pattern; two transcriptional units are coordinately regulated from a single control region and both code for enzymatic activities rather than there being a single operon for a regulatory factor and another for enzymatic activities. Other examples of divergent transcription are the *argECBH* cluster[45], the $\lambda p_R - p_{rm}$ system[46] and the *araCBAD* locus[47].

In both $\lambda p_R - p_{rm}$ and *ara*, the rationale for divergent transcription is to permit the modulation of autoregulation. In the $\lambda p_R - p_{rm}$ region, three operators overlap both back-to-back and overlapping promoters[46]. A low level of repressor both stimulates leftward transcription from p_{rm} to produce more repressor protein and decreases rightward transcription from p_R (ref. 14). As repressor accumulates, all the operator sites become occupied and transcription from p_{rm} is shut off. In *ara*, the *araC* gene product acts both as a repressor of its own synthesis and an activator of the arabinose catabolic operon in the presence of arabinose[47,48]. Again, the two operons are interactive, transcription of *araC* is not independent of transcription of *araBAD*[19]. Thus, in these operons, divergent transcription permits fine tuning of the primary metabolic operon and the operon coding for the autoregulated control protein. This is not the case for the *bio* cluster as none of the *bio* genes are required for the expression of remaining *bio* genes[9].

In summary, the *bio* regulatory region has been located by two independent methods. It is proposed that the *bioA* and *bioB* promoters are located face-to-face and partially overlap. Direct evidence that the promoters are in the proposed positions must come from sequence studies of individual promoter mutations or from the 5′ end sequences of the *in vivo* mRNAs because all *in vitro* binding or transcription assays are potentially subject to artefacts. It nevertheless seems unlikely that the same small DNA region that contains the site where insertion of IS*1* inactivates *in vivo* transcription of both *bioA* and *bioB*, and that possibly contains the *bioP98* and *bioO34* mutations, should coincidently contain the sites for the initiation of *in vitro* transcription. The proposed operator site contains or is entirely composed of an imperfect palindromic sequence. A new promoter may be created by the *bioP98* mutation.

Detailed descriptions of the RNA and DNA sequence determinations from positions −180 to +150 will be presented elsewhere.

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letters

Preferred frequency for interstellar communications

MANY scientists believe that intelligent life is common in our Galaxy[1–6] and that electromagnetic waves are the most practical means of communication[7,8]. Even equipment of a terrestrial standard can produce communication within a distance of 100 light yr. Several searches for interstellar signals have been proposed; however, difficulty arises in the selection of technical parameters such as frequency and coding. Among these, the selection of frequency is the most important. We propose here that the 4,829.659-MHz line frequency of the $1_{11} \rightarrow 1_{10}$ rotational transition of formaldehyde is a plausible frequency.

Simple calculations show that the number of possible frequency channels is so large that a search is not realistic, and we can assume that another party wishing to communicate would also consider this unpractical. Thus we need to predict the other party's decisions, and we can rely only on the common knowledge between the two parties. This also applies to the other party, so certain discrete frequency channels can be specified.

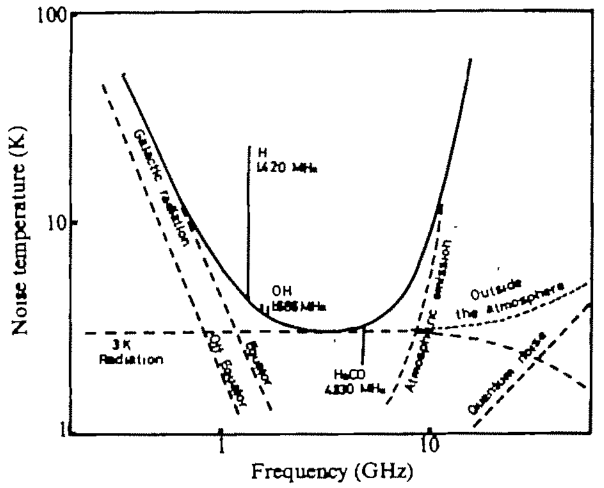

Fig. 1 The background noise temperature plotted against frequency. The solid line shows the total noise temperature on the surface of the Earth. Outside the atmosphere, the influence of atmosphere can be neglected in high frequencies and the total noise temperature is shown by the short-dashed line. The long-dashed lines show each component which sums up to form total noise emission. The absorption line at 4.83 GHz is the formaldehyde absorption band in the direction of dark clouds.

Table 1 Candidate stars in the direction of Coal Sack Nebula taken from the Michigan Spectral Catalogue

No	HD no	Spectral type	Photographic magnitude	Position (1900 0) RA	dec
1	109478	F0	9 2	12h 29.8min	−64°43′
2	109622	G5	9.9	12 30.9	−63 25
3	109699	K4/M0	9.6	12 31.5	−62 10
4	109811	F0	9 7	12 32.5	−64 47
5	109819	F8/G0	9.3	12 32 6	−62 22
6	110040	K5	10.3	12 34.2	−61 11
7	110310	F0/2	7.9	12 36.0	−64 11
8	110735	F6/7	9.0	12 39 0	−61 40
9	110830	F2/3	9.1	12 39.7	−61 38
10	111002	G0/2	9.9	12 41.0	−60 52
11	111455	G5/8	9 8	12 44.2	−61 54
12	111557	F3/5	9.3	12 44.9	−62 27
13	111580	F5	9.1	12 45.1	−64 22
14	111778	K0	10.0	12 46 5	−61 50
15	111905	F7/G0	10.0	12 47 4	−63 34
16	112109	F0	8.2	12 48.9	−63 6
17	112169	F2	9.3	12 49 4	−62 35
18	112216	G0/2	9.5	12 49.8	−62 4
19	112513	M2	10.2	12 52.1	−62 17
20	112636	G3	9.0	12 53.0	−62 23
21	112703	F5	8.0	12 53 5	−63 50
22	112752	K5	9.9	12 53.8	−64 32
23	112795	F3/6	9 7	12 54.1	−63 41
24	112854	G8	9.5	12 54.5	−62 7
25	113015	G0	10 0	12 55.6	−61 56
26	113152	F2	9.1	12 56.5	−60 47
27	113181	K5	11.2	12 56.7	−62 38
28	113182	K0	9.7	12 56 7	−62 56
29	113375	K3	9.5	12 58 1	−64 15
30	113466	G5	9 5	12 58 8	−63 42
31	113707	G3	10.3	13 0.4	−61 25
32	113824	F5	10.5	13 1.2	−61 4
33	113844	F8	10.0	13 1.3	−61 12
34	113860	F0	9.4	13 1.4	−63 28
35	114091	K5	10.2	13 3.1	−64 11
36	114252	F0	9.7	13 4 2	−61 18

Cocconi and Morrison[7] suggested the use of neutral hydrogen line frequency of 1,420.406 MHz which was then the only known radio spectral line in the Galaxy. If transmitted at this frequency, the possibility that the signal could be detected by chance by some unknown party is higher than at other frequencies: it would help to establish 'implicit mutual agreement'.

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The 4,829.659-MHz H_2CO line, because of the anti-maser effect, exhibits a very low excitation temperature[9]. Due to this anti-maser absorption, the background radiation temperature in the directions of dark clouds is below the 3 K background temperature. If a search is made at this frequency, we get a lower noise temperature than at any other frequency bands (the opposite is true for the hydrogen 21 cm line). Although the background noise temperature is lowered only by ~1 K, the depression itself could be interpreted as a significant marker.

A civilisation on a star which we see in front of a dark cloud must be aware of this advantage and would transmit signals at this frequency knowing that we know of the same advantage and could forecast their decision. One can specify the frequency of this absorption minimum within 5 kHz (300 m s^{-1} in Doppler shift) due to low temperature dark clouds. The absorption line is narrower than other lines, but above all, the frequency of the transmission signal can be set to the absorption minimum determined by observations and the search band is much narrower compared with other proposed bands. Properties of this frequency band as discussed above meet the requirements of the beacon signal necessary to establish the first contact. If intelligent civilisations are common throughout the Galaxy, they must have formed a community using communication and must be sending beacon signals.

There are many dark clouds, covering a substantial fraction of the sky and many stars that can be seen in front of such clouds. A characteristic of our method is that we can specify not only the frequency but also the directions to be searched.

Candidate stars in the direction of the nearest dark cloud, Coal Sack Nebula, are listed in Table 1. These are the stars of luminosity classes IV and V with the spectral types F, G, K and M in the Michigan Spectral Catalogue[10].

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The controversial carbon solid–liquid–vapour triple point

THE controversy over the solid–liquid–vapour (S–L–V) triple point of carbon began in 1849 (ref. 1). Since then, investigators have developed widely differing points of view which are discussed here. Some believe the triple-point pressure is $\sim 10^5$ Pa (~1 atm), whereas others believe it is $\sim 10^7$ Pa (~100 atm). Over the past 40 yr, the 10^7 Pa view has dominated but the evidence is not entirely convincing. In the past few years, new evidence has been obtained that apparently ends the controversy at least from the standpoint of the proper order of magnitude of the pressure.

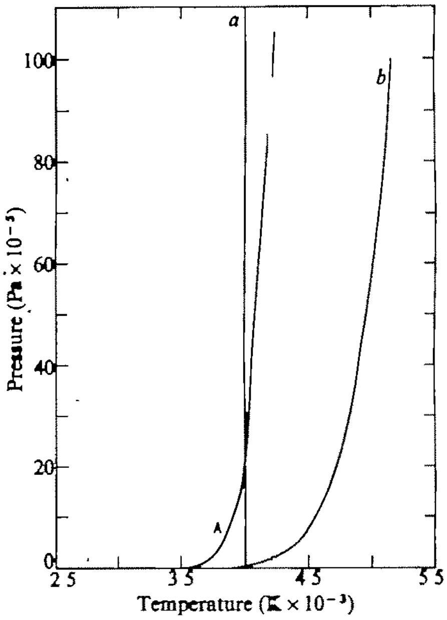

Fig. 1 Solid–liquid and solid–vapour boundaries for the low-pressure region of the carbon phase diagram. *a*, Bundy; *b*, JANAF.

In any single-component phase diagram, the S–L–V triple point is located at the intersection of the solid–liquid (S–L) and solid–vapour (S–V) boundaries. These boundaries for carbon are shown in Fig. 1. The S–L boundary was obtained by an extrapolation of Bundy's high-pressure data, and the JANAF data gives the S–V boundary[2,3]. Even though Bundy's extrapolated data could be in error by 100° or 200°, these boundaries (Fig. 1) indicate that the S–L–V triple-point pressure must be of the order of 10^5 Pa. However, the S–L–V triple point was believed to occur at $\sim 10^7$ Pa and 4,200 K, and it was considered that the JANAF calculations above ~3,000 K might be wrong. Thus, S–V boundaries, such as A in Fig. 1, were proposed, even though there was little experimental basis[4]. The question of which S–V boundary is correct can be answered by considering the results of several studies on the vapour pressure of carbon in the region of 4,000 K.

Covington, Liu and Lincoln[5] and Lundell and Dickey[6] have obtained vapour pressure data that confirmed the JANAF data in the region of 10^5–10^7 Pa. Also, Maurer *et al.*[7] obtained data at $\sim 10^4$ Pa that agreed with the JANAF curve. Data obtained several years ago indicated that graphite transforms to the more stable carbyne forms of carbon above ~2,600 K. Hence, the equilibrium S–V boundary must lie below the JANAF curve. All these data are shown in Fig. 2 in the region of the S–L–V triple point. I have recently reported[11] preliminary data on the rate of transformation of graphite into each of two of the carbyne forms[11]. These data also show that the lowest transformation temperature is ~2,600 K and supports the conclusion that the equilibrium vapour pressure of carbon must be less than the JANAF values above this temperature. These rate data show that the transformation graphite → carbyne is relatively slow up to 2,800 K (first-order rate constant of $\sim 10^{-5}$ s at 2,800 K). There is some evidence that the transformation rates increase with temperature as expected, but 2 or 3 s are required for 80–90% conversion in the region of 3,800 K. Therefore, the transformation did not have time to take place in the experiments of Covington, Liu and Lincoln because their measurement time was of the order of 1 ms or less. Hence, their data would be expected to fall on the JANAF curve because it represents the vapour pressure that graphite would have if it were the stable form above ~2,600 K. In the experiments of Lundell and Dickey, it is difficult to estimate the time required to heat and vaporise the carbon because the beam traversed through the sample at an appreciable rate. However, it seems

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that the time available for transformation of the carbon was well below 1 s, especially at the higher power densities. Therefore, their data would be expected to fall on the JANAF curve. These five groups of data constitute a mutually consistent set that experimentally establishes the S–V curve in the region of 4,000 K and rules out the possibility of an S–V curve in Fig. 1A. With the S–V curve thus established, the only other way to get an S–L–V triple point at $\sim 10^7$ Pa would be for Bundy's data to be in error by $>1,000$ K, which is highly unlikely.

If the S–L–V triple-point pressure of carbon occurs at $\sim 10^5$ Pa, $\sim 10^5$ Pa, then it is reasonable to question the significance of the results obtained at $\sim 10^7$ Pa. The pressure reported range from 1.01 to 1.22×10^7 Pa, but most values approximate $1.01 - 1.03 \times 10^7$ Pa. Temperatures range from ~ 3,970 to 4,390 K with no tendency to cluster about a particular value. All these high-pressure results have at least two major shortcomings: (1) in all cases, it was assumed that the carbon vapour pressure equilibrated with the pressurising gas. This does not necessarily happen, and some means must be provided to show that this condition has been achieved. Therefore, the pressures are suspect as they are not really experimentally determined but essentially assumed values. (2) No spectroscopy was carried out on the radiation that arrived at the pyrometer. It must be confirmed that line or band emission is not causing spurious pyrometer readings and it must be shown that the radiating gas surrounding the specimen is not optically dense. In the laser heating experiments of Whittaker *et al.*, much spectroscopy was carried out on the solid and gas phases. It was found that the carbon gas emits only Swan radiation. If impurities are present, additional lines or bands appear in the spectrum; hence, it is important to ensure that the pyrometer uses a wavelength range that is free of this radiation. Also, the optical density of the gas was found to increase with pressure, and, at $\sim 10^6$ Pa, the effect of the optical density is no longer negligible. At 10^7 Pa, the optical density would be quite high. Hence, a pyrometer may not be able to 'see' the surface of the condensed phase at this pressure. In this case, the reported temperatures would have little meaning. Indeed, the effect of high optical density could account for the wide range of reported temperatures.

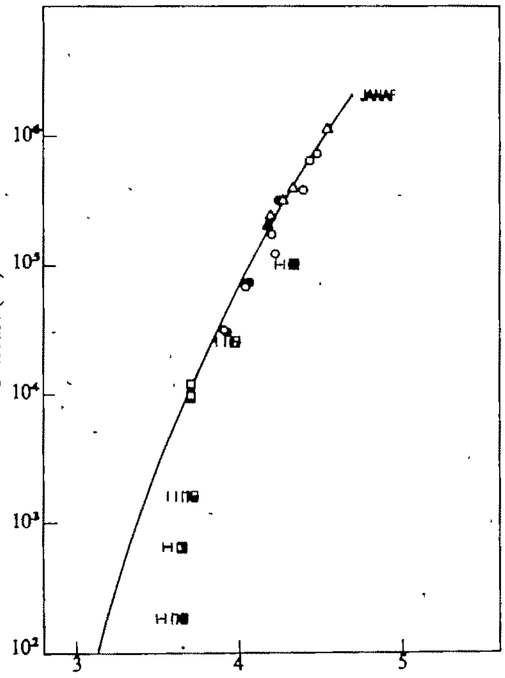

Fig. 2 Vapour-pressure data for carbon in the region of the S–L–V triple point. △, ref 6; ⊙, ref. 5; ⊡, ref. 7; |·| ( ), refs 8–10, with error bars and number of determinations shown.

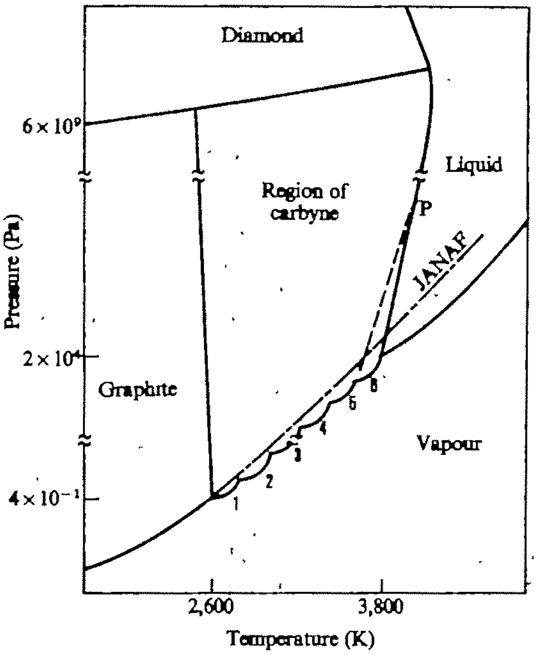

Fig. 3 Proposed carbon-phase diagram[10].

One possible reason that the high-pressure data clusters in a general region is that a solid–solid–liquid triple point could exist near 10^7 Pa at point P in the proposed phase diagram (Fig. 3)[10]. In this case, the solid phases would have to be carbyne forms of carbon. As the equipment used in the high-pressure work was not designed to study condensed-phase equilibria, the observed pressure and temperature would be some kind of average determined by the competition between the condensed-phase equilibrium and the liquid–vapour equilibrium attempting to coexist. This situation would result in a large scatter in reported temperatures and pressures because this competition would depend on the idiosyncrasies of each particular apparatus.

The uncertainties discussed here make it difficult to interpret the high-pressure results. However, the recent vapour-pressure data together with Bundy's high-pressure results, contribute to the conclusion that the S–L–V triple point of carbon occurs at a pressure of the order of 10^5 Pa, and that the reported results in the region of 10^7 Pa must refer to some other phenomenon.

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A surface related component of iron metal in lunar samples?

SOLAR wind elements implanted into lunar soil grains come to rest within a few hundred angstroms, consequently high concentrations should build up in near surface locations. It has become common practice to 'identify' surface correlated species by investigating sieved soil fractions; the increased concentrations in fine grain sizes being attributed to the greater surface area. The approach used[1–4], infers a surface location for finely divided iron metal (Fe(0)) and associated hydrolysable carbon (C_{hyd}) in lunar samples. These studies, together with other evidence[5], imply solar wind induced reduction processes were involved in producing metal from ferrous iron (Fe(II)) in silicates. The experiments described here performed with a reagent ($CuCl_2/2KCl$), which should only attack exposed metal, have failed to recognise a genuine exposed surface component. We believe that some effect associated with solar wind sputtering is responsible for iron formation and we must therefore question the validity of the model on which the initial conclusions in favour of surface located metal were based.

To obtain a quantitative measure of surface located iron metal, a mild specific leaching agent was used. Although X-ray photoelectron spectroscopy (XPS)[6,7] may distinguish between Fe(II) and Fe(0) at depths <20 Å, this technique is not quantitative. Fe(0) is readily dissolved in a $CuCl_2/2KCl$ solution due to the replacement reaction[8,9]:

$$Fe^0 + Cu^{2+}(aq) \rightarrow Fe^{2+}(aq) + Cu^0$$

Two gram aliquots of tachylyte basaltic glass (Snake River, Idaho) together with 0–5 mg quantities of iron metal (high purity, British Chemical Standards) were treated with 25 ml of an aqueous solution of the reagent (13.33 g $CuCl_2 \cdot 2H_2O$, 11.66 g KCl in 250 ml doubly distilled water); filtered solutions were diluted so that iron was in the 0–1 p.p.m. range and analysed by atomic absorption to provide a calibration graph. A comparison with results obtained by acid treatment of iron turnings only, suggests that the copper reagent dissolves Fe(0) completely within the error limits of the measurement. The copper solution does not leach Fe(II) from the basaltic glass. Milligram aliquots of three suites of size differentiated glassy agglutinates separated from lunar soils 15601 and 12023 by sieving and density–magnetic methods[2,10] were treated with 2 ml quantities of reagent. After hand shaking intermittently during a two hour period or standing over night, the liquid was directly aspirated into the flame of the atomic absorption spectrometer. Figure 1 compares the measurements of the amount of Fe(0) taken into solution with the total abundance of metal (diameter <130 Å) determined by magnetic susceptibility[2,10]. With the exception of one sample of 15601 (150–250 µm fraction), the iron abundance in solution is small, close to the background or detection limits of the analytical method. There is no relationship with particle size; the single positive result could be attributed to dissolution of an iron meteorite fragment(s). The reagent is capable of dissolving Fe(0) associated with the samples since a variable quantity, up to 20% of the total, is found in solution after fresh $CuCl_2/2KCl$ solution is added and agitated by ultrasonic vibration for 15 min (only sample 12023 $M > 3.5$ is shown in Fig. 1). Even after ultrasonic treatment, the amount of Fe(0) taken into solution does not increase with decreasing particle size; if anything our limited data suggest the opposite.

We interpret the above results as follows: the vast majority of Fe(0), possibly >99% of the total, is located in sites inaccessible to the reagent and not on the very surface of the samples studied. It could be argued that metal was present in cavities, vesicles and pore spaces[11] which could not be penetrated by the liquid. The analysis of solutions obtained using ultrasonics, however, are not in keeping with such a hypothesis, as very fine fractions do not release the largest quantities of metal even after prolonged agitation. A microscopic examination of 12023 samples following ultrasonication shows that the agglutinates have been broken up; probably the iron was exposed to the reagent as a consequence. We would expect large agglutinates to be more fragile than small ones. For some reason, not yet clear, 15601 agglutinates were not disrupted by ultrasonic vibration.

Several mechanisms have been proposed for the production of lunar Fe(0) metal (for a review see refs 11 and 12). The hypotheses currently receiving the most attention involve reduction by some aspect of sputtering and by definition produce metal only at the very surface of grains. At face value, the results we have presented apparently preclude sputtering. They seem to favour a chemically based mechanism, whereby reduction is induced by meteorite impact heating, because water is formed from silicate oxygen and solar wind hydrogen atoms[13]. This latter mechanism might generate surface correlated metal which was inaccessible to the copper reagent. There is very strong evidence that a physical (sputtering) rather than a chemical reduction is involved: (1) both preferential sputtering of oxygen atoms[6] and preferential deposition of heavy elements, particularly iron[11], are possible in practice; preferential sputtering is theoretically predictable[12]: (2) XPS and Auger spectroscopy demonstrate major elements at particle surfaces are fractionated relative to their abundance measured for the interior[7,14]; and (3) silicon and oxygen at grain surfaces are depleted in light isotopes relative to these elements at greater depth[15]. Because of the apparent paradox, it is necessary to reconsider whether increased abundance of Fe(0) in fine fractions may only be interpreted as the presence of a surface related component.

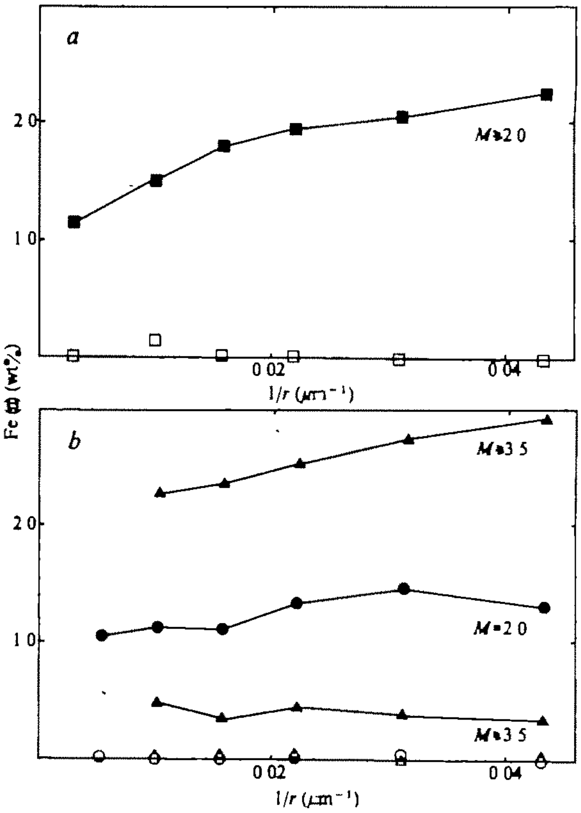

Fig. 1 Metal content of sieved fractions of glassy agglutinates from lunar soils: a, 15601; b, 12023. Closed symbols (■, ▲, ●) are for magnetic susceptibility measurements, open symbols (□, △, ○) are for $CuCl_2/KCl$ leach determinations and ▲ refers to material agitated by ultrasonic vibration whilst in the liquid reagent. The values $M = 2.0$ and $M \geq 3.5$ are the setting on the magnetic separator used to obtain the agglutinates from bulk soil; here they only identify the different fractions.

Figure 1 shows the data plotted as c against $1/r$ (where c = concentration and r = average radius of a size fraction). According to one model[16] a surface component is recognised from the slope of the line; the volume related component can be determined for particles of infinite radius from the intercept on the y axis. In this model the volume related component is assumed to be constant for all grain sizes. Such an assumption is invalid because lunar soils contain many, in fact sometimes a majority of, grains which are complex aggregates, either glassy agglutinates or microbreccias. The volume related component is due to the incorporation of a surface component into the interior of complex particles. There is no reason to suppose a volume component produced in this way would be constant for all grain sizes. On the contrary, it might be expected that fine agglutinates and breccias, which must be made up of constituent fragments with a smaller median grain size than their coarse counterparts, ought to have a larger volume related component due to the increased surface incorporated. A volume component which increases in fine fractions would be indistinguishable from a surface component on a plot of c against $1/r$. An alternative model[17] which considers $c \propto d^{-n}$ (where d is diameter) and recognises surface and volume related components from a plot of $\log c$ against $\log d$ is also incapable of distinguishing a surface from an increasing volume component.

We now contend that surface related components of Fe(0) and C_{hyd} are minimal; c against $1/r$ or $\log c$ against $\log d$ plots recognise increasing volume related components, which are a result either of a greater abundance of aggregated particles or a greater complexity within such grains in fine fractions. Some support for the latter interpretation has come from detailed analysis of progressively more magnetic agglutinates from lunar soil 12023 (ref. 10). We have pointed out[2,12,18] that genuine surface components of $Fe(0) < 130$ Å in size would be unlikely to survive in the terrestrial atmosphere and that only metal incorporated into agglutinates would be protected from oxidation. Agglutinate metal behaving as an apparent surface related component because of increases in volume concentration does not conflict with such arguments.

If model predicted surface components of Fe(0) and C_{hyd} are in fact volume components, what proportion of the apparent surface correlated solar wind hydrogen, rare gases, carbon and nitrogen can be accounted for by this effect? The answer to this question presumably will come from comparison of data from size fractions with measurements made by surface specific techniques such as ion microprobe[19,20] and nuclear reactions[21,22]. Calculations[23] and direct measurements[22] suggest maximum surface carbon concentrations are up to a factor of five less than estimates made on the basis of $1/r$ plots[24] and thus support arguments in favour of an increasing volume component.

We are not denying the existence of surface components, only questioning their magnitude; surface components must exist to be incorporated into complex grains to generate volume related components. We need better models to describe the accumulation of solar wind elements and solar wind derived species in lunar soil. Experiments using surface specific techniques to detect genuine surface components are highly desirable.

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Organic ^{14}C activity in an abyssal marine sediment

ONE measure of transport rates and residence times of organic carbon within the various organic carbon pools in the ocean is to follow perturbations from the 1952–58 and 1961–62 atmospheric thermonuclear bomb tests on the natural ^{14}C activities of living, detrital and dissolved organic matter. We report here our measurement of the ^{14}C activity of the total sedimentary organic carbon (SOC) in an abyssal red clay, as no such data were available for this organic carbon pool. Ultimately, these sedimentary organic ^{14}C activities may be compared with the ^{14}C activity of the source organic material in the water column and estimates made of the magnitude of organic carbon consumption at the seawater–sediment interface. What was found, unexpectedly, was evidence for the penetration of recent organic carbon from the 4 cm mixed layer at the sediment–seawater interface down to at least 12 cm in the sedimentary column. This suggests a different and more rapid mechanism for the sedimentation of some fraction of the SOC other than direct association with clay minerals.

Photosynthetic fixation of bomb-derived $^{14}CO_2$ in the euphotic zone and its subsequent incorporation into bathypelagic marine fish and crustacea and into deep-sea particulate and dissolved organic matter has been previously studied in the north Central and North-east Pacific Ocean[1–4]. This work (Table 1) showed that the dissolved (plus colloidal) organic carbon (DOC) had 'apparent ages' of 740 yr BP at the surface to 2,570–3,470 yr BP at 2,000 m, and that the ^{14}C activity of the particulate organic carbon (POC) at 2,000 m was 10% less than its activity at the surface. These observations are indicative of slow recycling of DOC between the surface and deep waters and rapid sinking of POC (or some fraction of it) in the water column. Thus, high ^{14}C activities of SOC in the upper mixed layer and at the seawater–sediment interface would reflect this rapid sinking of POC to the sea floor coupled with its relatively slow biochemical oxidation to CO_2 by benthic populations.

The 0–30 cm sediment sections (Table 2) were sub-sampled from two box cores[5] (0.25 m² in area) taken on 10 July 1970 (core 33) at 6,040 m in the central North Pacific Gyre (30°0.9′ N, 156°12.5′ W) and on 8 June 1977 (core 638) at 5,780 m in the same area (28°34.4′ N, 155°30.3′ W). This region is part of an extensive abyssal plain situated beneath low-productivity surface waters. The sediment–seawater slurry (equivalent to a

Table 1 Natural radiocarbon activities of various carbon pools in the central and eastern North Pacific Ocean

Pool	Depth (m)	Date	Location	$\Delta^{14}C$ (‰)	Age (yr BP)*
POC	10	1971[3]	30°00′ N 140°00′ W	$+237 \pm 24$	—
POC	2,000	1971	,,	$+182 \pm 27$	—
DOC	10	1971	,, ,,	-88 ± 20	740 ± 170
DOC	500	1971	,,	-176 ± 14	$1{,}560 \pm 140$
DOC	2,000	1971	,,	-274 ± 13	$2{,}570 \pm 140$
DOC	1,880	1968[1]	30°16′ N 119°49′ W	-351 ± 27	$3{,}470 \pm 330$
DOC	1,920	1969[1]	30°16′ N 119°50′ W	-341 ± 23	$3{,}350 \pm 280$
DIC	5,292	1973†	31°22′ N 150°01′ W	-219.5 ± 3.5	$1{,}991 \pm 46$
SOC (this report)					
Core 33 (0–3 cm)	6,040	1970	30°01′ W 156°13′ W	-664 ± 22 (mean)	$8{,}760 \pm 510$ (mean)
Core 638 (0–3 cm)	5,782	1977	28°34′ N 155°30′ W	-736 ± 20	$10{,}700 \pm 630$

POC, particulate organic carbon; DOC, dissolved organic carbon; DIC, dissolved inorganic carbon; SOC, sedimentary organic carbon.

*Ages calculated relative to the NBS oxalic acid standard activity using the Libby half-life (5,568 yr). Since pre-nuclear bomb surface seawater carbonates were 4% lower in activity ($\Delta^{14}C = -40$‰), all the ages given in this report are about 340 yr younger using this correction.

†Pacific Geosecs, Leg 1, Station 204 (H. Östlund, personal communication).

2-mm vertical section) was sucked-up from the undisturbed centre (25 cm × 26 cm area) of core 33 and sieved through a 65-μm mesh stainless-steel screen. The solid upper 3 cm of this core was then scraped into five arbitrary sections. The upper 3 cm of core 638 was sliced into 1-cm sections only as the surface of this core was slightly disturbed. The remainder of each core was sampled in 2-cm increments down to 30 cm leaving about 5 cm of sediment on the bottom. The samples were then frozen at −20 °C for subsequent analysis. All feasible precautions were taken to prevent contamination of the samples with ^{14}C or dead carbon. The analytical results and a summary of experimental procedures for each analysis are given in Table 2. The $\delta^{13}C$ values are included to show that they fall within the range of marine organic-$\delta^{13}C$ analyses (−18 to −22‰ for subtropical waters).

Sedimentation rates below the mixed layer for the clay mineral fraction of cores 33 and 638, determined by $^{230}Th/^{232}Th$ activities, were 1.0 and 1.2 mm 10^{-3} yr^{-1}, respectively (Fig. 1*a*). It is clear from this plot that the depth of the mixed layer is 8.0 ± 1 cm (core 33) and 4.0 cm ± 0.5 cm (core 638), assuming uniform mixing within the layers. The 4 cm mixed layer thickness for core 638 is corroborated by the total SOC analyses (Fig. 1*b*). If SOC is laid down at the same rate as the clay minerals, then no measurable ^{14}C activity would be expected much below the mixed layer, however, significant ^{14}C activity was found down to at least 11–13 cm in core 638 (Table 2, Fig. 2).

The ^{14}C activity in the surface slurry (0–0.2 cm) from core 33 was not significantly higher than the ^{14}C activity found at 0.2–0.4 cm, and just significantly greater than the ^{14}C activity in the 2.7–3.0-cm section (Table 2). This implies rapid mixing and/or rapid utilisation in the mixed layer of POC reaching the seawater–sediment interface. The presence of bomb-^{14}C in the mixed layers in cores 33 and 638 can be seen by calculating a hypothetical mean age of the SOC in the mixed layer[9] assuming the $\Delta^{14}C$ value of the source organic carbon before the bomb tests was 0‰ (see Table 1). This calculation is based on the thickness of the mixed layers, M (8.0 and 4.0 cm), the $^{230}Th/^{232}Th$ sedimentation rates, S (1.0 and 1.2 mm 10^{-3} yr^{-1}) and the decay constant for ^{14}C, λ (1.2449×10^{-4} yr^{-1}) where the mean age $= 1/\lambda \ln[1 + (\lambda/S)M]$. For core 33 this calculated mean age is 19,200 yr and for core 638 it is 13,200 yr (vertical dashed line, Fig. 2). When these calculated ages are compared with the experimentally determined ages (Table 2, Fig. 2), it is apparent that more bomb ^{14}C has entered the mixed layer in core 33 than in core 638. This increased input of bomb ^{14}C in the mixed layer of core 33 is also reflected by the 42% more $^{239+240}Pu$ that was found in the 0–3-cm section of core 33 than in the 0–3-cm section of core 638 (Table 2). The presence of $^{239+240}Pu$ activity in the mixed layer of both cores is evidence of the rapid sedimentation of high ^{14}C activity POC as the flux of $^{239+240}Pu$ is, in part, through association with sinking organic detritus[10].

The main feature of the organic ^{14}C activity in the sediments is the presence of measurable ^{14}C activity below the mixed layer in core 638 (Fig. 2). An 'apparent sedimentation rate' of recent SOC, calculated from the ^{14}C activities at 6, 8 and 12 cm is 1.15 cm 10^{-3} yr^{-1}, and 0.95 cm 10^{-3} yr^{-1} if calculated from the activities at 1.5, 6, 8, and 12 cm (Fig. 2). These rates are about 10 times those derived from $^{230}Th/^{232}Th$ ratios, but due to uncertainties in the ^{14}C activity determinations, the recent SOC rates could vary from 0.8 to 6 cm 10^{-3} yr^{-1}. Thus, the introduction of recent organic carbon below the mixed layer is

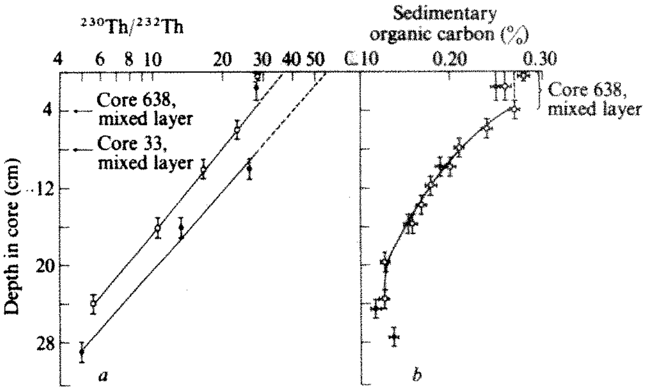

Fig. 1 *a*, $^{230}Th/^{232}Th$ ratios plotted as a function of core depth: ●, core 33; ○, core 638. Error bars denote one standard deviation. The lines are least squares fits and the depth of the mixed layers are indicated by arrows. The clay mineral sedimentation rates below the mixed layer are 1.0 mm 10^{-3} yr^{-1} for core 33 and 1.2 mm 10^{-3} yr^{-1} for core 638 where the half life of ^{230}Th is 7.52×10^4 yr. *b*, Total sedimentary organic carbon (SOC) expressed as per cent of dry weight and plotted as a function of core depth: ●, core 33; ○, core 638. Error bars denote one standard deviation. The dashed line from 0 to 4 cm is the mean of values at 0–1, 0–3 and 3–5 cm for core 638.

independent of the sedimentation rate of the clay minerals or SOC associated with them.

The mechanism or mechanisms responsible for this apparently anomalous injection of significant quantities (calculated to be 7–13% of the total SOC depending on whether the source carbon is pre-bomb, $\Delta^{14}C = 0‰$, or post-bomb, $\Delta^{14}C = +180‰$) of recent SOC into the sedimentary column below the mixed layer is not clear. One explanation is organic carbon transport by burrowing organisms such that the numbers and lengths of their excursions, and hence the organic carbon flux, decreases with depth. The long straight burrows, 10–30 cm in length, which have been observed in abyssal sediments[11] are due primarily to worms burrowing into the sediment. The worms pump in surface seawater, filter out organic matter and eventually retreat leaving organic tubules behind. This mechanism for organic carbon transport is questionable for two reasons: (1) the smooth distribution of thorium isotopes (Fig. 1*a*) do not reflect any large scale bioturbation, and (2) the number of burrows normally observed in deep sea sediments do not seem sufficiently dense[12,13] to maintain the necessary flux of recent organic carbon.

A second explanation for the injection of organic carbon into the sediments is diffusion of recent DOC into the sedimentary column from the mixed layer. This mechanism would require a negative gradient of DOC in the interstitial water and a high concentration of DOC to be continuously added to the mixed layer by relatively rapid biochemical processes (solubilisation of detrital POC, autolysis of organisms, and excretion of DOC by organisms). However, the opposite mechanism is indicated by an analysis of DOC in the interstitial water of a pelagic clay from the central equatorial Pacific[14] (10°40′ N, 147°40′ W) where the DOC concentration increased from 2.5 mg C l^{-1} at the surface to 4.8 mg C l^{-1} at 30 cm in this core. Because the amount of DOC in the interstitial waters of red clays is only 0.1–0.4% of the total SOC, unrealistically high diffusional fluxes of DOC would be required even with strong, negative DOC gradients. Thus, neither of the above mechanisms satisfactorily explains the presence of recent organic carbon 6–12 cm below the mixed layer in this pelagic red clay.

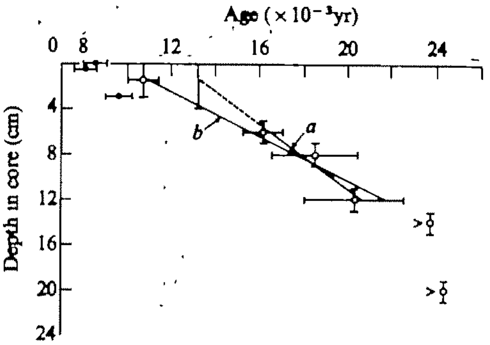

Fig. 2 The ^{14}C activity of the sedimentary organic carbon plotted as ages (yr BP) against core depth: ●, core 33; ○, core 638. Error bars denote one standard deviation (Table 2). The symbol (>) means that the ^{14}C-organic carbon activities at 13–15 and 19–21 cm were within 2σ of background and hence error bars were not applicable. The lines *a* and *b* are least squares fits for points at 1.5, 6, 8 and 12 cm; and at 6, 8 and 12 cm, respectively. The vertical dotted line from 0–4 cm represents the calculated age of the sedimentary organic carbon in the mixed layer for core 638 (see text).

There is some corroborative information on possible bioturbation below the mixed layer from $^{239+240}Pu$ analyses of two box cores collected on the 1977 cruise. These two cores were taken 1.6 and 6.4 km from core 638. The $^{239+240}Pu$ analyses (V.

Table 2 Measurements made on the sediments and sedimentary organic matter from box cores 33 and 638

Sections (cm)	SOC (%)	H_2O (%)	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	Age (yr BP)	$^{230}Th/^{232}Th$	$^{239+240}Pu$ (d.p.m. kg^{-1})
Core 33 (1970)							
Surface slurry (0–0.2)	0.20		−19.7	−655±22	8,500±500		
0.2–0.4	0.30		−19.7	−635±23	8,100±500		
2.7–3.0	0.27		−18.7	−703±22	9,700±600		
0–3		57				27.3±0.1	1.7±0.08
9–11	0.19	51				26.0±1.3	
15–17	0.16	50				13.2±0.4	
24–26	0.12	49					
28–30	0.14	49				4.9±0.2	
Core 638 (1977)							
0–1	0.28	53				27.8±2.2	
0–3	0.26	53	−20.1	−736±20	10,700±600		1.2±0.16
3–5	0.27	52				23.0±1.8	
5–7	0.24	51	−18.3	−866±15	16,200±900		
7–9	0.21	51	−20.3	−900±21	18,500±1,900		
9–11	0.20	50				17.6±0.7	
11–13	0.18	49	−21.0	−920±20	20,300±2,200		
13–15	0.17	50	−21.1	−948±0	>23,700		
15–17	0.16	49				10.2±0.2	
19–21	0.13	49	−21.3	−952±0	>24,300		
23–25	0.13	50				5.6±0.3	

The thawed sediment samples used for ^{14}C activity determinations were acidified to *p*H 3–4 with distilled 6 M HCl, and all samples were dried to constant weight at 80 °C. Thereafter, the dried sediment samples were burned at 800–900 °C for 2–3 h in a stream of prepurified oxygen. Conventional vacuum line techniques were used to obtain CO_2 of high purity necessary for gas-proportional counting. Each CO_2 sample was aged for 4 weeks before counting to ensure decay of any ^{222}Rn (3.8-d half life). The ^{14}C activity was measured using a 100 ml quartz gas counter filled to a pressure corresponding to 90 cm CO_2 at 25 °C. In these conditions, 95% of the activity of the NBS oxalic acid standard was 0.727±0.015 c.p.m. above a background of 0.815±0.012 c.p.m. A minimum of 10,000 counts was accumulated for each sample, normally over two separate counting periods. Two blanks were run; one using no sediment and the other using an oxidised portion of the original sample. No CO_2 was evolved by either procedure. The CO_2 recoveries were 70–90% based on the total SOC contents. The $\Delta^{14}C$ and $\delta^{13}C$ values and ages quoted in Table 2 have been calculated in the normal manner[6]. The $^{230}Th/^{232}Th$ (ref. 7) $^{239+240}Pu$ (ref. 8) methodologies were applied to the same non-acidified, dried sediment samples as used for the ^{14}C determinations, and the SOC contents were done using a Hewlett-Packard CHN Analyser. No differences were found in SOC between acidified and non-acidified sediment samples within the error of the method (±0.015% C), indicating that the carbonate content of the sediment was <0.03%. This was confirmed by infrared analysis of the CO_3^{2-}–CO_2 in non-acidified sub-samples.

Bowen, personal communication) showed penetration of $^{239+240}$Pu down to 12 and 14 cm in these cores. Previous measurements[15] by Bowen for two box cores taken in 1974 in the same general area showed penetration of $^{239+240}$Pu from the surface down to 5 and 8 cm in the sedimentary column.

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Did emerging continents trigger metazoan evolution?

A DIVERSE fauna of trilobites, archaeocyathids, and other shelly invertebrates can be found in rocks of early Cambrian age, but in strata antedating the Cambrian such skeletonised remains are notably absent. This seemingly abrupt appearance of multicellular animals seemed illusory to Darwin[1], whose vision of evolution assumed the gradual transmutation of species over innumerable generations. His view of life necessitated a long Precambrian record of evolving Metazoa and he, therefore, attributed the apparent absence of Proterozoic fossils to the imperfection of the fossil record and the vagaries of sedimentary geology. Discoveries of the past two decades have shown the first metazoans to be less ancient than Darwin believed. It now seems that the Metazoa evolved only in the latest Precambrian (perhaps 750–650 Myr ago), and that 100–150 Myr of evolution was sufficient to delineate the various phyla in evidence at the base of the Cambrian. The question of whether emerging continents controlled the late appearance of multicellular animals on Earth is discussed here.

Hargraves' theory[2] of crustal evolution has inspired new hypotheses on metazoan origins. Hargraves believes that the primitive Earth had a thin, but continuous, sialic crust blanketed by a world ocean some 2.8 km in depth. As the Earth matured, mantle activity progressively built the sialic crust into continental masses. The thickness of these continents was controlled isostatically, the critical crustal depth being the 750 °C isotherm (the temperature at which granitic crust begins to melt). As long as the Earth's interior was hot and, consequently, the 750 °C isotherm was shallow, the continents could not become thick enough to rise above sea level. Only as the Earth cooled could continental blocks emerge from and dissect the world ocean. Hargraves sets wide limits on the timing of continental emergence. His geophysical model for terrestrial cooling predicts that continents should have appeared 1,400–900 Myr BP, while his estimate of sedimentary rock volumes through time suggests that the actual time of emergence was 900–600 Myr BP. Hargraves does, however, acknowledge that certain patterns of sedimentation are most consistent with the existence of emergent continents before 1,700 Myr BP.

Chamberlain and Marland[3] were the first to consider the biological implications of Hargraves' theory. If Hargraves is correct, they reasoned, then before the buildup of continents to within 200 m of sea level, the global sea would have been stratified. Nutrient recycling by upwelling would have been minimal, and consequently, organic productivity would have been low. Chamberlain and Marland link both increased algal productivity and metazoan evolution to the appearance of persistently emergent continents.

LaBarbera[4] has taken these arguments further and developed an explicitly hargravesian theory of metazoan origins. He argues that it was the emergence of continents in the late Precambrian that triggered the evolution of multicellular organisms. Before the end of the Proterozoic era, environments in which early invertebrate benthos could proliferate simply did not exist, except during a few transitory periods.

If we wish to accept the theories of metazoan evolution implied by Chamberlain and Marland and defined by LaBarbera, we must first examine the physical model on which they are based. Instead of judging the merits of the Hargraves' model in its entirety[5], I shall concentrate on the aspect that bears directly on the derivative biological arguments—the timing of the appearance of continental land masses. If sizeable continents with their associated implications for erosional runoff, shallow marine platform environments, and upwelling currents existed significantly before the end of the Precambrian, then it is difficult to attribute the evolution of Metazoa to their appearance. The appropriate data for the examination of this question are the preserved accumulations of Precambrian sedimentary rocks. From these strata, one can derive important information about the type, depth, and extent of depositional environments, as well as data relevant to the delineation of sediment source areas.

The Proterozoic sedimentary record of North America is relatively well known and illuminates the problem. The oldest Proterozoic sediments on this continent are those of the Huronian Supergroup exposed north of Lake Huron. This 2,300 Myr old sequence, which has an aggregate thickness of ~15,000 m near the southern limits of exposure, contains a variety of features suggestive of deposition on the margin of a large, emergent land mass: deposition on a pre-Huronian erosion surface, sedimentary structures indicative of fluvial deposition, other structures referable to tidal flat environments, clasts derived from a granitic source area to the north, and a set of characteristics (striated pavement and boulders, tillite, and dropstones) that record an episode of early Proterozoic glaciation[6–9]. Sediments of similar age and character are also known from other areas of North America: the Kaminak Basin, North-West Territories; the Chibougamau Basin, Quebec; and the Libby Creek Group of Wyoming[10].

Sedimentary rocks deposited between 2,200 and 1,800 Myr BP are common in North America, occurring in linear geosynclinal belts and as the remnants of broad platform sequences.

Goodwin[11] has shown that the geographical distribution of these deposits is consistent with the occurrence of two large, stable early Proterozoic cratons separated by a metastable cratonic area. Goodwin's continental areas are bordered on all sides (within the limits of existing outcrops) by miogeosynclinal sedimentary rocks. Within these facies, there is abundant physical evidence of shallow water sedimentation. For example, in the miogeosynclinal belt of the Coronation Geosyncline, North-West Territories one finds arkose and orthoquartzite (derived from a source located in the direction of Goodwin's hypothesised craton), oolites, mudcracks, and gypsum casts[12]. Similar features characterise the proximal facies of the CircumUngava Geosyncline in eastern Canada[13–15]. Contrary to the assertion by LaBarbera, stromatolites abound in early Proterozoic carbonate sequences. The abundance and variety of stromatolitic structures in Coronation[16] and Circum Ungava[17,18] shelf sediments, as well as in other coeval deposits (see Table 1), provide strong testimony to the wide distribution of shallow marine environments 2,000 Myr ago. These environments were not the transitory products of dynamic crustal features such as island arcs. They are thick sequences resting on stable Archean granites and gneisses. In short, it is difficult to account for the observed pattern of early Proterozoic sedimentation without postulating the existence of large emergent or periodically inundated continental masses.

Table 1 Occurrences of early Proterozoic stromatolites

Geological unit	Location	Refs
Gunflint/Biwabik Fms	Lake Superior, USA and Canada	46, 47
Kona Fm.	Michigan, USA	48, 49
Great Slave Supergroup	North-West Territories, Canada	50, 51
Hurwitz Group	North-West Territories, Canada	51
Sutton Lake Group	Ontario, Canada	51
Manitounuk Group	Quebec and Labrador, Canada	51
Belcher Group	Belcher Islands, Hudson Bay, Canada	13–15, 51
Mistassini Group	Quebec, Canada	52
Vallen Group	Southern Greenland	53
Mt Bruce Supergroup	Western Australia	54
Fortescue Group		
Hamesley Group		
Wyloo Group		
Frere Fm.	Western Australia	55
Batchelor Group	Northern Australia	30
South Alligator Group	Northern Australia	30
Ventersdorp Group	South Africa	56
Transvaal Supergroup	South Africa	26
Lomagundi Dolomite	Rhodesia	57
Karelian Series	Soviet Karelia, Finland	58
Burzyan Group	Southern Urals USSR	59
Upper Dharwar and Aravalli Groups	India	60

The mid-Proterozoic history of North America strengthens the case for the early appearance of continents. Between 1,700 and 1,000 Myr BP, several grabens developed at various times and in various parts of the Canadian Shield[19–21]. These basins were filled with continental redbeds and basalts in a way similar to that characterising the Triassic–Jurassic rift basins of eastern North America. In the North-west Territories, one also finds evidence of a >1,500 Myr old blanket sandstone (the Thelon Formation and its equivalents)[19] that seems to be similar in nature to the Phanerozoic 'Nubian' Sandstones of Africa. Palaeocurrent analysis of this formation suggests sediment derivation from an actively eroding land area in the vicinity of Hudson Bay[11]. During this same period thick sequences of clastics and stomatolitic carbonates were accumulating on the eastern (Grenville)[22] and western (Belt/Purcell)[23] shelves of the shield. Again, a large, emergent craton seems to be fundamental to any explanation of mid-Proterozoic sedimentation patterns in North America.

The North American record is not unusual in its pattern of Proterozoic sedimentation. Indeed, data from other continents corroborate the picture of Precambrian continents inferred from North American rocks. Several episodes of epicontinental and continental sedimentation covered the Kaapvaal Craton of southern Africa during the early Proterozoic era[24]. In earliest Proterozoic times, auriferous fluvial and alluvial clastics of the Witwatersrand Supergroup accumulated in a continental basin whose area was at least 70,000 km^2 (ref. 25). Stromatolitic carbonates of the overlying Transvaal Supergroup (2,200 Myr) suggest a shallow marine platform of still greater dimensions. Eriksson and Truswell[26] have traced the Malmani Dolomite throughout an area of 210,000 km^2. (Not only is this formation widespread geographically, it is also up to 1,500 m thick.) The Transvaal sequence itself is overlain by an extensive blanket of redbeds belonging to the Waterberg Group (1,800 Myr BP)[27].

Similar patterns can be found in Australia where broad platforms and geosynclines characterise early[28–30], middle[30,31], and late Proterozoic[32,33] sedimentary sequences. Palaeogeographical maps of the USSR for the early Riphean (1,650–1,350 Myr) illustrate emergent Russian Platform and Siberian shield areas flanked by broad expanses of shallow marine platforms[34]. In South America, deltaic sediments are known from the earliest Proterozoic of Brazil[35], and 1,800–2,000 Myr old blanket sandstones are widely distributed across the Guyana Sheild[36].

From the geological evidence, it seems that the observable record of Proterozoic sedimentation does not support the thesis that continents first became emergent late in the Precambrian era. Sizeable cratonic areas have been a persistent feature of the Earth for at least the past 2,300 Myr (I do not argue here that continents emerged at this time or that they ever emerged as envisioned by Hargraves, I simply state that they were there 2,000 Myr ago). The contention that continental areas existed in the early Proterozoic does not carry with it the implication that Hargraves' graph of sediment volume against time is necessarily incorrect. The apparent increase in sediment volume indicated for the Phanerozoic could be a real phenomenon related to Phanerozoic plate movements. Geological evidence—including cross-bedded quartz arenites, stromatolites, tidal flat sequences, and shales whose geochemistry suggests prolonged subaerial weathering—indicates that emergent land masses and shallow marine environments also existed in the Archean, although the extent and longevity of such features are less well defined than they are for the Proterozoic (see refs 37–39). I cannot agree, therefore, that Metazoa arose in response to the initial appearance of land on the Earth. LaBarbera's hypothesis that the first primitive herbivores and deposit feeders evolved from a pelagic planuloid ancestor is plausible, as is his contention that the subsequent evolution of predation greatly increased the complexity of the food web and led, through increasingly specialised adaptations, to the diversification of multicellular animals. But these steps need not have been triggered by emerging continents. They could equally well have taken place in pre-existing shallow water environments.

The concept that it was the removal of some physical barrier that made metazoan evolution possible is not new. A decade ago, the Berkner–Marshall hypothesis[40] gained widespread popularity because it provided a simple set of non-biological controls over biological innovation[40]. But the physical constraints of oxygen and ozone, like those of habitat, seem to have been removed well before the first invertebrates populated the ocean floor[42].

Why then did the Metazoa evolve when they did? I believe the answers are fundamentally biological in nature, rather than physical. As hypothesised by other workers, the rapid evolution of multicellular animals at the end of the Precambrian may have resulted from a combination of biological factors including the origin of eukaryotic sexuality, the ecological consequence of cropping, and the acquisition of that most basic of metazoan characteristics, a biochemical mechanism for the functional differentiation of genetically identical cells[43–45].

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Identification of ancient heat treatment in flint artefacts by ESR spectroscopy

IT is of archaeological importance to be able to determine whether or not a particular sample of flint has been subjected to heating, either intentionally or accidentally, during its utilisation by man. We report here on the use of ESR spectroscopy for the study of flint, and show that heated material can be readily identified by the appearance of a characteristic ESR signal which is absent from the unheated flint. This signal is considered to be stable over an indefinite period of time. We have also explored other implications of this technique in the examination of flint, including its possible use for dating purposes.

Worked flint is the most abundant source of evidence for human activity before the advent of pottery-making cultures, and any information which can be deduced from it regarding its technology and date of fabrication is of considerable archaeological interest. Excavations frequently produce flint which has been accidentally subjected to the action of heat in a fire, but it is now recognised that from at least the Solutrean (~17000 BC) some flint was intentionally heated to improve its working properties[1,2]. Using experimental fabrication technique[3,4] and examining artefact material[5] show that the technique of pressure retouching is facilitated in certain flint samples by controlled heat treatment of the material, and that this fact was known and exploited in antiquity. Samples thus treated can often be recognised by the greasy lustre of fractures produced after heating, but because the useful heat treatment temperature is in the region of 300 °C, well below that at which gross structural changes become apparent, it is important to have a reliable method for identifying flint which has been heated. Flint which has been heated above about 380 °C may be suitable for dating by the thermoluminescent dating method[6,7], and some dates have been obtained for archaeological samples although there are still considerable problems associated with the application of this technique to flint which are not yet resolved. Thermoluminescence (TL) has been used to determine prehistoric heat treatment of chert artefacts[8], and has proved successful for material heated within the past 10,000 yr. Caution must, however, be exercised with samples from earlier sites where the TL acquired since heating approaches the saturation value for the unheated geological material, and the method is necessarily destructive.

The technique of ESR spectroscopy has been used to assess radiation damage in geological materials[9] and has recently been applied to cave deposits and bone associated with early human activity[10], but it has not previously been used for artefacts. It is generally less sensitive than TL but can, in principle, lead to firmer identification of the radiation-induced centres.

Our results on unheated geological samples showed the presence of a very narrow readily saturated line ($g = 2.0017$, width ($\Delta H_{MS} = 1.0$ G), which we assign to trapped electrons (e_t^-). On heating to 400 °C for 15 min this signal was lost, and exposure to a 100 krad dose of ^{60}Co γ-rays at 300 K readily restored it. We conclude that qualitative study of this e_t^- signal could be a useful and complementary technique to TL in studies of flint artefacts, and we are attempting to establish quantitative links.

Most significantly, on heating, a new signal (C) ($g = 2.0035$, $\Delta H_{MS} = 3.5$ G) developed. This is characteristic of amorphous carbon[11,12]. Traces of organic matter are known to be present in flint[13], and we postulate that these pyrolyse on heating to give carbon. The paramagnetic defects responsible for the ESR signal are, as far as we know, infinitely stable at ambient temperatures. Hence the presence of a C-signal should be diagnostic of heat treatment. To test this, we studied a group of upper Palaeolithic flints (~20000 BC), two of which were thought on the basis of appearance to have been heated in antiquity. The former gave clear C-radical signals together with e_t^- features, whereas the latter gave only the e_t^- signal.

These results establish that we have discovered a well defined method of assessing the occurrence of heat treatment in archaeological flint samples. We are now initiating a quantitative study of the C-signal as a function of the firing temperature, the time of firing and the nature of the flints. Hence we hope that ESR studies can establish conclusive details of heat treatment, and may also help to establish the probable date of such treatment.

We have also detected various other ESR signals in certain flint samples, some of which are characteristic of transition metal ion impurities such as Mn(II) and Fe(III). The form of the spectra provide information about the local symmetry around these ions. By studying their response to exposure to ^{60}Co γ rays, we should be able to gauge the extent to which these impurity ions contribute to radiation effects in flint. This could be significant as their participation could greatly modify TL in such samples.

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Botanical evidence linking the New Zealand Maoris with New Caledonia and the New Hebrides

TRADITIONAL history of the Maoris tells of repeated voyages between Hawaiki, their mythical homeland, and Aotearoa (New Zealand), culminating in a considerable migration about AD 1350 dated by genealogies. One canoe is said to have brought seed of the karaka tree which was a useful subsidiary food source. I report here that examination of the distribution and relationships of this tree suggests New Caledonia and/or the New Hebrides as the possible Hawaiki from which this late migration originated.

The tree *Corynocarpus laevigatus* J.R. et G. Forst. is commonly seen in the North Island of New Zealand and in the warmer coastal parts of the South Island to the southerly limit of Banks Peninsula. It is well known by its Maori name *karaka*, or on Chatham Islands where it is also commonly known by the Maori name *kopi*. Over its whole range along the coastline frequent groves are explained as Maori plantings marking old villages or campsites because in pre-European times the tree was important for food. The large kernels are poisonous but became valuable and highly nutritious when steamed or baked and washed to free them from the compound karakin which causes severe convulsions and usually death if eaten[5].

Among the interesting stories of the ancient traditional history of the New Zealand race[11], there is the record that karaka berries were brought to New Zealand in the canoe Aotea, one of a group or 'fleet' supposed to have brought a large number of Maori immigrants about the year AD 1350 (ref. 3). Other Polynesian settlements had been established in New Zealand several centuries before this wave of colonisation[3,7]. Botanists, nevertheless, have described the tree as an endemic species and it is generally regarded as such today[1], although its distribution is not as widespread as might be expected had it been growing in New Zealand for millions of years.

Seed grows readily and is widely planted in gardens throughout the country. In the wild wherever plants are protected from stock which eat foliage and fruit, seedlings arise in profusion around old trees, although they do not all survive if heavily shaded. Native pigeons, *Hemiphaga novaeseelandiae*, are known to feed on ripe berries and probably void viable seeds which are protected by a hard coat, although pigeons in laboratory trials were killed by a small dose of extract of karakin or its hydrolysate[2]. Occasional seedlings which have been noted some kilometres away from coastal groves may have been established by pigeon distribution, although pigeons are feeble fliers and if they retained seed for long they risk being poisoned. Maoris also planted seed along ridges and bush routes which they traversed, and groves may be found near old settlements growing from discarded seed thrown on middens[13].

Cockayne, discussing the vegetation of New Zealand[4], describes *C. laevigatus* as a leading physionomic plant in coastal vegetation but also makes the important remark. "As Maoris were accustomed to plant this tree near their villages doubt must frequently arise as to its occurrence in certain localities not being due to this cause. Few plants spread more rapidly by means of seed so its presence in apparently primeval forest does not disprove its being absent in the primitive community." More detailed observations on the distribution of the karaka tree are necessary, but the firm impression remains from wide observation over its range that the species is absent from many places where it can grow very well, and where one would expect it to be were it truly indigenous. The distribution on the Kermadec Islands[15] seems to be sporadic as it is in New Zealand, as though the Maoris introduced it rather than took it from there, as has been suggested[3].

Hemsley[12] described another species of *Corynocarpus* from the New Hebrides as *C. similis* because it closely resembled *C. laevigatus*. A re-examination of this species has led me to the opinion that *C. similis* is in fact the same species as *C. laevigatus*. Two flowers of the holotype of *C. similis* can be matched in detail with flowers of *C. laevigatus*, especially in the structure and toothing of the staminodes which Hemsley used as a distinguishing character. Additional flowering specimens of *C. similis* collected in the New Hebrides and fresh flowering material collected on the island of Efate are all in agreement. Leaves of the New Hebridean plants have a slightly larger maximum size and are distinctly thinner, but venation, epidermis and the finely down-rolled margin correspond with New Zealand specimens. Inflorescences are also more open and lax. Such vegetative differences may warrant making the New Zealand and New Hebridean plants different varieties of the same species, but the exceedingly close relationship between the two is certain and obviously is of special interest in connection with the Maori tradition of the origin of the karaka in Hawaiki. Hemsley[12] also described a new species, *C. dissimilis*, from New Caledonia. Careful examination shows flowers and foliage of this to be so close to material of *C. laevigatus* that it is assumed to be conspecific.

It seems improbable that the natural distribution of this single species should cover the New Hebrides, New Caledonia and New Zealand, in the light of distribution of other species of the New Zealand flora and the highly endemic character of its trees. The heavy, bulky, poisonous seed sinks in water, so that recent natural distribution over such a distance seems to be ruled out. *C. similis* is known to be present on all the main New Hebrides islands and has a vernacular name on each, seeming, therefore, to be a truly endemic plant of the group: none of the vernacular names listed by Gowers[9] bears any resemblance to the Maori names karaka or kopi. The genus is unknown from Fiji and Samoa, which are both well explored botanically. One other distinct species extends from Malaysia to Queensland[14].

Some early link between the New Hebrides, New Caledonia and New Zealand is strongly suggested by this botanical evidence, for some trees are clearly very old. As they do not form annual rings age cannot be counted in the usual way. Yen[16] has written of the movement of the sweet potato, *Ipomoea batatas*, from South America to Oceania, and the survival of the Peruvian, Ecuadorian and Bolivian name kumara. Although direct evidence of the people who effected the transfer has been lost, it is generally considered that they were probably Polynesian voyagers making early contact with South Americans between AD 400 and 700. If ocean travellers had collected the karaka in a similar manner it should be present throughout Polynesia in places other than just the New Hebrides, New Caledonia and New Zealand. This distribution supports the suggestion of direct migration, from New Caledonia and/or New Hebrides to New Zealand, based on two-way voyaging which would have suggested that an otherwise perhaps unimportant food plant would be of more use in the new land.

New Zealand anthropologists today discount the story of the 'fleet'[10], but the older suggestion that this traditional history has a factual basis is still accepted by many, including myself. Although it is clear that the links of the earlier Polynesian settlers who arrived in New Zealand from about AD 900 are all with east Polynesia[3,7], the evidence of the karaka suggests that later Polynesian settlers who arrived about AD 1350 came from New Caledonia and/or the New Hebrides, which are presumed to have contained Polynesian settlements, about 600 yr ago. They are now inhabited by Melanesians, although Polynesians and Polynesian languages are found in the New Hebrides. The large area of these islands and their fine timber resources could have supported a considerable population of seafaring people. Instructions in the traditional history speak repeatedly of the necessity of steering to the east when sailing from Hawaiki to Aotea (New Zealand). The expert seamanship and navigational ability of the Polynesians, appreciated by Captain James Cook and all the early European navigators in the Pacific, have been well examined recently[6,8] and can be in little doubt, although questioned by some[10]. The fighting referred to in the traditional history, which seems to have been a main factor driving the people of the later migration out of their Hawaiki, may have been with Melanesian invaders ousting the Polynesians, who fled to New Zealand in considerable numbers taking with them such western cultural features as the rich use of curvilinear designs and the production of many kinds of weapons, which are so characteristic of the later 'classical' Maoris but are absent from the Moa-hunter period.

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Humeral morphology of the earliest apes

KNOWLEDGE of the skeletal anatomy of early hominoids has been based on various skeletal parts of fossil apes from the Miocene of East Africa and Europe. Postcranial remains from the earlier apes of the Oligocene of Egypt consisted of a single ulna, a basal phalanx and a hallucial metatarsal[1–4]. During 1977 a palaeontological expedition from the Cairo Geological Museum and Duke University recovered four new partial humeri attributable to these Egyptian apes. These specimens now provide the first evidence of humeral anatomy of Oligocene apes and permit a better assessment of primitive forelimb morphology and locomotor abilities of earliest hominoids.

All four fossils were recovered from quarries in unconsolidated channel sands of the 'Upper Fossil Wood Zone', Jebel Quatrani Formation. This formation has been radiometrically dated as >26 Myr old[5]. All four bones are undistorted but broken so that they preserve varying portions of the distal third of the humerus. Each humerus is distinctly recognisable as primate, and because of differences in absolute size they seem to represent two taxa. Two of the specimens (DPC-1026, CGM-40123) are similar in size to humeri of the extant New World howling monkey *Alouatta*. These bones are almost certainly attributable to the largest of the Egyptian Oligocene apes, *Aegyptopithecus zeuxis*. Each articulates well with the previously described ulna of this species[1]. Two other humeri (DPC-1033, DPC-1045), although similar in overall morphology to the larger fossils, are from a smaller animal the size of the bearded saki (*Chiropotes*) (Tables 1 and 2). There are two ape species smaller than *Aegyptopithecus* known from the quarries which yielded these humeri, the type species of *Aeolopithecus*, and a new undescribed species of *Propliopithecus*. It is not clear to which of these smaller species, if either, the latter two humeri are attributable.

In order to assess both their functional and their phylogenetic significance, the new fossil humeri have been compared with samples of over 30 species of living primates and a variety of nonprimate mammals using both metric and qualitative characters. Table 2 compares the Oligocene humeri with selected groups of extant species using indices which reflect the most distinctive features of the fossils.

Table 1 Characteristics of three humerus specimens of Oligocene apes

Specimen	DPC-1026	CGM-40123	DPC-1033
Biepicondylar width	34.1	—	23.4
Angular projection of medial epicondyle	8.6°	—	6.2°
Ventral articular width	24.5	22.9	17.0
Ventral trochlea width	11.5	11.3	8.2
Ventral capitulum width	12.5	11.5	8 9
Maximum capitulum depth	13.0	11.8	10.1
Olecranon fossa depth	5.5	5.6	4.3

All linear measurements are in millimetres.

In the form of the distal articular surfaces, the fossil humeri show none of the derived features found among modern cercopithecoid monkeys and lack many derived humeral features characteristic of extant hominoids. Rather, they show a more primitive mosaic of features similar to those found in Miocene fossil apes and some present-day New World monkeys. As in most primates, the capitulum is rounded rather than cylindrical, a shape which presumably permits extensive rotation of the radius about the ulna during pronation and supination. The surface of the capitulum extends well onto the inferior surface of the humerus, indicating that the early apes were capable of a considerable range of elbow extension[6]. Compared with the condition seen in cercopithecoids, the radial side of the humerus

Table 2 Comparison of Oligocene humeri with extract species

	Capitulum depth	Trochlea width	Medial epicondyle		Olecranon depth
Species	Articular (Index *A*)	Capitulum (*B*)	Articular (*C*)	Angular projection of medial (*D*)	Articular (*E*)
Chiropotes satanas (2)	0.614	0.614	0.271	44.0°	0.307
Alouatta sp. (7)	0.579	0.809	0.365	20.4°	0.196
Macaca fascicularis (7)	0.825	0.642	0.258	44.1°	0.392
Cercopithecus aethiops (7)	0.848	0.648	0.256	50.1°	0.422
Cercopithecus mitis (6)	0.789	0.661	0.288	42.6°	0.352
Presbytis obscura (3)	0.710	0.774	0.212	37.0°	0.373
Hylobates sp. (7)	0.692	0.868	0.295	6.0°	0.304
Pan troglodytes (5)	0.623	0.946	0.400	15.8°	0.346
Dendropithecus macinnesi (1)	0.575	0.920	0.342	0°	0.311
Pliopithecus vindobonensis (1)	0.524	0.842	0.314	0°	0.253
DPC-1026	0.530	0.920	0.351	23.0°	0.224
CGM-40123	0.515	0.983	—	—	0.245
DPC-1033	0.594	0.921	0.364	24.0°	0.252

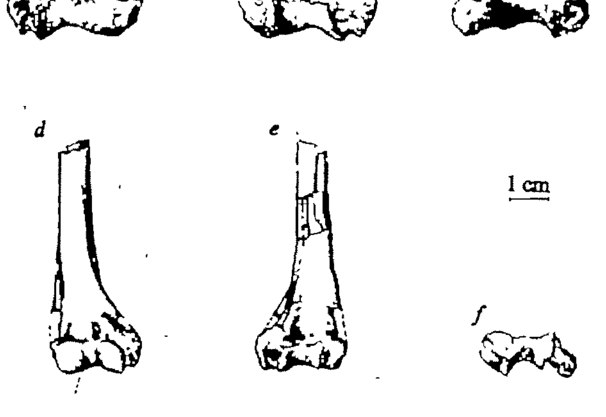
Values of the indices are given as means.
Figures in parentheses represent the number of specimens measured.

is relatively shallow in the new fossils as in New World monkeys and other fossil hominoids (Table 2, index *A*).

Medially, a low ridge separates the capitulum from the trochlea. In these Oligocene apes, this ridge is much less marked than that found among living hominoids or in Miocene *Dryopithecus africanus*; it is more comparable in shape to that of the Miocene ape *Pliopithecus* or of larger cebids such as *Alouatta*. These humeri show the relatively broad trochlea found among all other extant and fossil hominoids. This feature separates them quite clearly from cercopithecoid monkeys (Table 2, index *B*). Since a broad trochlea is also found among many prosimians, as well as these Fayum specimens, its presence among modern apes is almost certainly a retention of a primitive condition.

The Oligocene humeri lack the distinctive spool-shaped trochlea with pronounced lateral and medial margins that characterises extant apes and the Miocene fossil ape *D. africanus*. This spool-like shape, Jenkins has argued, is biomechanically adapted for maximising elbow stability through an extensive range of flexion and extension as well as in pronation and supination[7]. Even so, these fossils do not possess a narrow trochlea with pronounced anteromedial and posterolateral borders such as is characteristic of cercopithecoid monkeys (and many other pronograde quadrupedal mammals)[7]. The broad relatively shallow trochlea of Fayum apes, with its slightly developed anteromedial lip, is more comparable to that found among the more generalised arboreal quadrupeds such as the large cebids *Alouatta* or *Chiropotes*. In these New World monkeys the elbow is frequently flexed and the forearm semipronated during quadrupedal locomotion[8].

Fig. 1 Anterior (*a*), posterior (*b*), and distal (*c*) views of the left humerus of *Aegyptopithecus zeuxis* (DPC-1026) Anterior (*d*), posterior (*e*), and distal (*f*) views of the right humerus of a smaller hominoid (DPC-1033).



In the Fayum ape humeri, there is a large medial epicondyle which projects slightly posteriorly (Table 2, *C*, *D*). Among extant apes and many prosimians this process is large and projects directly medially. Inward projection of the medial epicondyle increases the medial rotatory torques which the digital and carpal flexors, as well as pronators, exert about the radio-humeral and ulnar-humero joints[7]. Amongst most Old World monkeys the medial epicondyle projects more posteriorly than medially as an adaptation for enhancing the actions of the pronators and flexor muscles when the elbow is already pronated as in pronograde quadrupedalism. The condition found among the Fayum apes is similar to that occurring in the larger arboreal quadrupedal cebids such as *Alouatta* or *Lagothrix* and represents a biomechanically intermediate condition between that of Old World apes and of cercopithecoid monkeys. On the dorsal surface of the medial epicondyle is a pronounced dorsal epitrochlear fossa similar to that seen in *Alouatta* and *Apidium*[3].

As in most extant prosimians and cebids, the olecranon fossa of Oligocene apes is quite shallow (Table 2, index *B*). In pronograde quadrupeds and knuckle-walking apes the olecranon fossa is deep. In function, a shallow olecranon fossa primarily appears to reflect the reduced posterolateral lip on the trochlea, and hence a reduced need for ulnar-humero stability during full extension of the elbow[7].

Although only one specimen preserves more than the most distal part of the humeral shaft, all four humeri show an entepicondylar foramen. This foramen, which transmits the median nerve and brachial artery from the dorsomedial surface of the arm to the cubital fossa, is characteristic of most prosimians (except lorises) and many cebids. Among catarrhines it is known only in the parapithecid genus *Apidium*[3], the Miocene hominoid *Pliopithecus*[9] and as a rare variation in other genera including *Homo*. Its occurrence in the Fayum hominoids confirms that its presence is the primitive condition for both higher primates and hominoids.

In all four humeri, there are indications of a prominent brachialis flange similar to that found among extant and fossil hominoids, as well as in medium-sized cebids and many climbing mammals. This flange is greatly reduced in Old World monkeys.

Few regions of the skeleton are more diagnostic for differentiating living monkeys and apes than the elbow region. The specimens described here provide new and unique palaeontological evidence about the morphology of this region in the earliest apes, that can be used to distinguish primitive from derived morphological conditions in living Old World higher primates. The new fossils indicate that a large medial epicondyle, relatively broad trochlea, and a pronounced brachialis flange, all found in Fayum apes, in *Apidium*, in many platyrrhines and prosimians as well as extant hominoids are primitive characters. The development of the distinctive spool-shaped trochlea with a strong ridge delineating its lateral border is a derived condition unique to extant hominoids. Such features of present day Old World monkeys as their relatively narrow trochlea with pronounced anteromedial and posterolateral lips, and relatively short, posteriorly-directed medial epicondyle are absent among the Fayum apes and parapithecids and represent derived conditions for cercopithecoids.

Thus, the new fossil evidence indicates that relative to the earliest known catarrhines, both modern apes and modern cercopithecoids show derived morphologies in the elbow region. To characterise early fossil hominoids as 'monkey-like' or 'ape-like' is to pose a false dichotomy. Rather, what can be seen in the humeral anatomy of the Fayum hominoids (as well as in many other aspects of the skeleton and cranium of these animals) is a distinct and more primitive morphology comparable to that found among the third group of living higher primates, the New World monkeys. Functionally, both the ulna[1,3,10] and humerus indicate that *Aegyptopithecus* was a largely quadrupedal, arboreal animal and that the best models for locomotor behaviour of early hominoids are neither the extant apes nor the extant cercopithecoid monkeys. Instead, closest resemblances are with the larger arboreal quadrupeds among platyrrhines, in particular the howler monkey *Alouatta*, which seems to demonstrate today a forelimb morphology similar to that exhibited by Oligocene apes.

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Induction of monozygotic twinning in the mouse

VINCRISTINE SULPHATE (Oncovin, Lilly) is a drug commonly used in the chemotherapy of acute leukaemias in children, lymphomas and certain other malignant conditions. We describe here experiments investigating the teratogenicity of this drug during the early stages of embryogenesis in the mouse. We report that a low incidence of gross anomalies was observed in fetuses on days 10 and 12 of gestation when pregnant females were treated with a single dose of this agent on the sixth, seventh or eighth day of gestation. However, contrary to expectation, a high proportion of litters from females treated on the seventh and eighth day of gestation contained at least one set of monozygotic twins. In this respect the present study is unique in that an experimental method of inducing a low but significant incidence of identical twinning in a mammal is reported. Furthermore, this work provides direct evidence regarding the comparatively late stage of embryonic development when identical twin formation may be induced in the mouse, as the most advanced stage at which twinning could be induced with vincristine was at the early headfold stage. However, the highest incidence of monozygotic twinning was obtained when pregnant females were treated on the morning of the seventh day of gestation, when embryos would be expected to be at the advanced egg-cylinder stage. It was in this latter group that a single conjoined twin of the janiceps type (cephalothoracopagus) was obtained. The possible aetiology of monozygotic and conjoined twin formation is briefly discussed here in the light of these experimental findings.

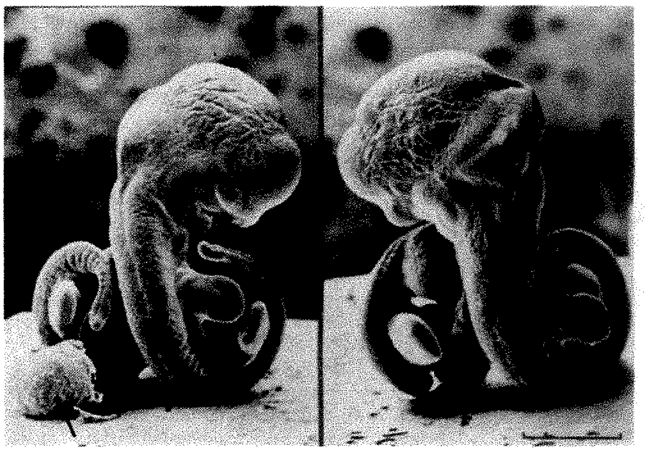

Fig. 1 Scanning electron micrographs showing two views of conjoined twin isolated on the twelfth day from a female injected with vincristine sulphate on the seventh day of gestation. A single head and thorax and two complete vertebral columns may be seen. A small round mass of tissue (arrowed) was attached to the umbilical region of one of the fetuses by a cord containing blood vessels. Scale bar, 1 mm.

Female mice, 6–8-week old virgin CFLP (Anglia), were mated with males of the same strain, and isolated on the morning of finding a vaginal plug (designated the first day of pregnancy). Females were injected intraperitoneally with 0.3 mg per kg vincristine sulphate in 0.2 ml normal saline on the morning of the sixth, seventh or eighth day of gestation. Controls were injected with a similar volume of normal saline. Autopsies were carried out on either the tenth or twelfth day of gestation. The uterine contents were examined, and the number and location of resorption sites and normal and abnormal fetuses recorded (Table 1).

In the only anomalous fetuses encountered, the region overlying the fourth ventricle was apparently collapsed, giving this region of the hindbrain an abnormally flattened appearance. These fetuses were otherwise apparently quite normal and healthy at the time of autopsy. It is not immediately clear whether the neural tissue in the hindbrain region of this comparatively small population of fetuses would have developed into, for example, an encephalocele which would have been recognisable at a later stage of gestation. It is also unclear whether these fetuses would have survived to term or into the early post-natal period. A higher incidence of fetuses with abnormal heads of this type was observed in the population of fetuses isolated from females treated on the seventh day (6.2%), compared with those isolated from females treated on

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Table 1 Incidence of resorptions, normal and abnormal fetuses and monozygotic twins in female mice treated with vincristine

Treatment	Day of injection	Day of autopsy	Total females examined	Implantation sites Living fetuses	Resorptions	Abnormal heads	Sets of twins
Vincristine	6	10	3	39	1	1	0
	7	10	5*	64	1	5	3
	7	12	4	48	3	2	1 (conjoined)
	8	10	4	52	1	1	1
	8	12	2	25	1	1	0
Saline	6	10	3	37	1	0	0
	7	10	4	51	2	0	0
	8	10	3	38	0	0	0

Female mice were injected with vincristine sulphate or saline (controls) on the sixth, seventh or eighth day of gestation.
* An additional two litters contained only resorbed embryos.

the sixth (2.6%) and eighth (2.6%) day of gestation. This low incidence of easily recognisable neural tube pathology is of interest in the light of earlier observations of Joneja and Ungthavorn[1], who investigated the effect of an intraperitoneal injection of this agent in mice on the ninth day of pregnancy. In addition to an increase in the incidence of resorptions, these workers observed that out of 77 fetuses examined at term 5.2%, 11.7% and 6.5%, respectively, were either microcephalic, exencephalic, or had an encephalocele. This difference may be related to the strain of mice used, or more probably to the later time of administration of this teratogen. Similar central nervous system anomalies have also been reported when hamsters were injected with this agent on the eighth day of gestation[2].

Another observation that seemed to be related to the time of exposure of pregnant females to this agent in the present series was the incidence of monozygotic twins. Four sets of twins, one pair of which was conjoined, were observed when females were treated on the seventh day of pregnancy. Of the five females autopsied on the tenth day, three each had one set of twins, and a single pair of conjoined twins (Fig. 1) was isolated from one out of four females autopsied on the twelfth day of gestation. One additional set of twins was obtained from a female treated on the eighth day. At least three of the twins shared a common amniotic cavity. No twins were observed in the fetuses isolated from females treated on the sixth day of gestation. Apart from the conjoined twins all the other twin fetuses seemed to be morphologically normal.

The main interest in the present study lies in the experimental production of monozygotic twinning in the mouse, as the normal incidence in this and most other mammalian species is thought to be extremely low[3,4]. In fact, polyembryony is extremely rare and only thought to occur regularly in certain species of armadillo[5]. It is generally thought that monozygotic twins probably result from the fission of a single embryonic axis shortly after the blastocyst stage has been reached, most probably during the early post-implantation period[6,7]. It is therefore interesting that the period of embryogenesis that was most susceptible to the twinning stimulus provided by vincristine in the present study was from the advanced egg-cylinder stage to the early headfold stage of development, and it is at this latter stage that mouse embryos have a distinct head process and primitive streak[8]. Rarely, incomplete separation of these embryonic axes occurs, and results in the formation of conjoined twins. This seems the simplest explanation for the isolated examples of conjoined twins reported in this and in earlier studies in which pregnant hamsters[4,9], rabbits[10] and rats[11] were treated with teratogens, and as spontaneous occurrences in other species.

The remarkably few reports in the literature in which monozygotic and conjoined twins have been recorded as spontaneous events and following treatment of pregnant animals with teratogens seems to indicate that twins of these types were previously encountered only as isolated occurrences, unlike the present study, where they appeared to be a definite response to the vincristine treatment.

The present experimental approach thus allows for the first time detailed examination of the early stages in the development of monozygotic twins, at precise intervals after the twinning stimulus has been given. Although the exact nature of the twinning stimulus evoked by vincristine remains to be established, it should now be possible to investigate monozygotic twinning in relatively controlled conditions.

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Plasmid-induced loss of virulence in *Vibrio cholerae*

WE have previously reported that pathogenicity of *Vibrio cholerae* was suppressed by introducing P or V plasmids and that this effect was due to decreased toxin production[1]. This work has been extended to show that cells harbouring both P and V plasmids become attenuated and do not cause experimental cholera. Such attenuated strains are attractive candidates for live oral vaccines if it can be shown that the immunogenicity of plasmid-harbouring strains is not altered and that they remain stable. We provide here convincing evidence that the cells are not cured of their plasmids *in vitro* or *in vivo* and do not lose their immunogenic potential.

Strain KB9 served as the parent virulent strain. P and V plasmids were introduced in KB9; two strains, designated as KB9:PV and CD24, were isolated, apparently different in one respect—that P is not expressed in CD24. This is not surprising because P is often repressed by V (ref. 2). Strain 569B is a comparatively poor pathogen *in vivo* but produces significant amounts of toxin *in vitro*. CD23 was derived from 569B and characterised as having P and V plasmids. KB92 (Ogawa) and KB93 (Inaba) were virulent challenge strains. The properties of strain KB9, and the technique of introducing P and V plasmids, characterisation of plasmids, growth of vibrios, media and buffer have been described in our previous report[1].

The pathogenicity of vibrio strains was examined in the ileal loop of adult rabbits[3] and intra-intestinal infection of vibrios in infant rabbits[4]. 1–2 × 10^9 cells of KB9:PV and CD24 in 1 ml brain–heart infusion broth (Difco) were injected separately into the ileal loops of rabbits and the open intestine of infant rabbits. Neither diarrhoea nor death was recorded in the infant rabbits up to 48 h and ileal loops of the adult rabbits were without any accumulation of diarrhoeal fluid. KB9, on the other hand, caused death of the infant rabbits with 10^5–10^6 organisms and ileal loops were full of fluid. For each strain, eight infant rabbits and an equal number of adult rabbits were used. LD$_{50}$ values of these strains were estimated in Swiss mice[1] and found to be 9, 10^6 and 10^7 organisms for KB9, KB9:PV and CD24, respectively. These data suggest that plasmid-bearing cells of *V. cholerae* behave as attenuated strains.

As 569B released significant amounts of toxin in Syncase minimal medium[1], CD23 was grown in similar conditions. Assay of the toxin in the culture filtrate of CD23 gave negative results. This is consistent with our earlier observation[1] that P and V plasmids interfered with the synthesis or release of toxin.

PV strains underwent two successive passages in rabbit intestine. The loss of plasmids was never detected. These strains were subcultured several times in broth, exposed to acriflavine and sodium dodecyl sulphate, and maintained at 37 °C in broth for several weeks. Individual colonies were tested but none were found to have lost plasmids. A large number of agents which cure *Escherichia coli* of plasmids could not eliminate the sex factor P in *V. cholerae*[5]. In addition, we have evidence that when plasmids are transferred from one cell to another in conjugal mating, a copy is always left behind in the donor. Unlike *E. coli*[6], P^+ or V^+ donors remain P^+ and V^+ after mating. The experiment used was as follows: the donor was P^+, Str^s, and the recipient was a Str^R, *ile*$^-$ *val*$^-$ *arg*$^-$ *his*$^-$strain. After bacterial mating[7], the mixture was plated on minimal glucose salt medium to score the donor population, and on media supplemented with amino acids and streptomycin to score the recipient. Individual colonies were tested and it was found that all the colonies of the donor strain were P^+ and among the recipients >60% were P^+. A similar result was obtained with a V^+ donor; however, the efficiency of transfer of the V plasmid was very low. These results strongly suggest that plasmids are very stable in *V. cholerae*.

Immunogenicity was examined by immunising rabbits subcutaneously with KB9:PV and CD24. Table 1 indicates a good correlation between protection to challenge and vibriocidal antibodies in the serum. Both strains conferred good protection in Pittman and Feeley's mouse protection test[8], particularly against homologous challenge strains.

Table 1 Protective immunity and serum vibriocidal antibody titre in rabbits immunised with kB9·PV and CD24

Vaccine	Challenge strains	% Immunity to challenge	Vibriocidal titre (mean)
KB9:PV	KB92	74	3 × 10^5
	KB93	100	9 × 10^4
CD24	KB92	81	1 × 10^5
	KB93	77	8 × 10^4
Control	KB92	0	<50
	KB93	0	<50

Swiss albino rabbits weighting 1–1.2 kg were used at the time of immunisation. KB9:PV and CD24 were grown in brain–heart infusion broth and 2 × 1 ml of live culture (1–2 × 10^9 ml^{-1}) was administered subcutaneously to each rabbit at 21-d intervals. Groups of 8–10 rabbits were immunised. They were challenged on the 35th day after the second dose of vaccine and starved for 48 h before challenge. Immunity to challenge was assessed in ligated ileal loops[3] and expressed as reported previously[10]. Serum vibriocidal titres were determined according to Finkelstein[11] on serum samples collected 1 d before challenge.

Thus, we propose that plasmid-bearing strains like KB9:PV and CD24 may be used as live oral vaccines. These strains possess several other features of a vaccine strain[9] and these will be reported elsewhere.

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Accessory cell requirement for anti-IgM-induced proliferation of B lymphocytes

THE nature of the signals which B lymphocytes must receive so as to proliferate and differentiate are not yet completely understood. It is likely that B cells with different triggering requirements exist[1,2], and that B-cell proliferation and B-cell differentiation require separate signals[3–6]. Because of this heterogeneity in signalling, it has been difficult to define precisely what is necessary to induce the expansion and differentiation of a particular B-cell clone after its exposure to antigen. It has been suggested that direct antigen binding to specific membrane immunoglobulin initiates at least one of the necessary activation signals[7–9]. Further activation signals may come from T cells and/or macrophages[7–9]. We have been studying the nature of the stimuli required for the induction of B-cell proliferation. We report here that using soluble goat anti-mouse IgM as a surface Ig cross-linking agent, we have confirmed that interactions with surface Ig can induce the proliferation of at least a subset of mouse B cells[10–12]. In addition, we have found a requirement for radioresistant phagocytic adherent cells in the polyclonal stimulation of B cells by this antibody.

Figure 1 shows the goat anti-IgM-induced proliferation of CBA/Tufts spleen cells. Optimal stimulation of 5 × 10^5 lymphocytes was consistently seen with 1–5 μg of MOPC-104E affinity column-purified goat anti-MOPC-104E (μ,λ) and TEPC-183 (μ,κ). Although 10–50 μg of normal goat Ig per culture induced a slight amount of proliferation, the stimulation was less than twofold above background. This is in contrast to the 13-fold stimulation seen with 1.25 μg of goat anti-IgM.

Purified μ,λ (MOPC-104E) or μ,κ (TEPC-183) myeloma proteins were equally effective in abrogating the proliferative response induced by the optimal dose of goat anti-IgM[13]. As little as 0.5 μg per culture of MOPC-104E or TEPC-183 completely inhibited anti-IgM-induced, but not lipopolysaccharide (LPS)-induced proliferation. Up to 4 μg per culture of

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Table 1 ^{3}H-Thymidine incorporation expressed as $\bar{X}\Delta$ c.p.m. ± s.d. (stimulation index)

	2 μg goat anti-IgM		0.5 μg K-235 LPS		0.25 μg Con A	
Expt 1						
Normal spleen cells	112,632 ± 22,934	(12)	124,464 ± 6,788	(13)	418,281 ± 8,476	(40)
M-depleted spleen cells	5,500 ± 1,982	(3)	126,099 ± 1,936	(40)	306,206 ± 4,860	(96)
M-depleted spleen cells + normal peritoneal MØs	124,864 ± 4,961	(10)	89,697 ± 7,829	(7)	403,908 ± 4,084	(29)
Expt 2						
Normal spleen cells	127,299 ± 14,512	(12)	228,083 ± 5,789	(21)	503,516 ± 18,623	(45)
Spleen cells + 50 μg silica	34,606 ± 13,641	(6)	237,780 ± 2,227	(38)	436,632 ± 14,725	(69)

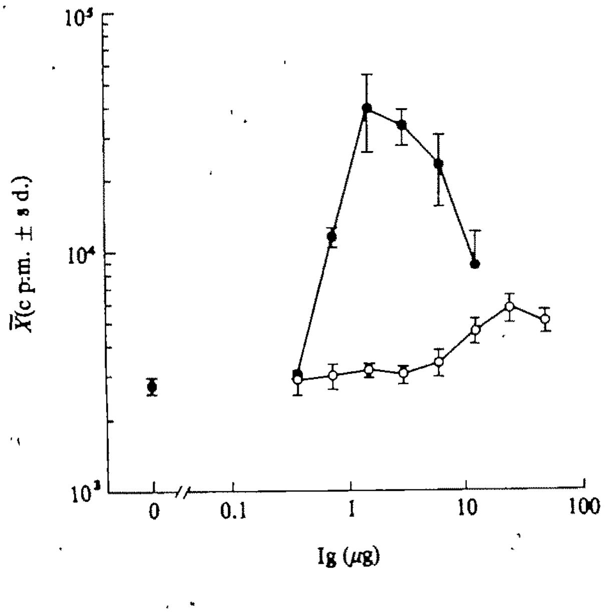
In expt 1, normal CBA/Tufts spleen cells were depleted of macrophages (M) by 2-h adherence to plastic Petri dishes in the presence of 20% fetal calf serum (FCS) and 2 subsequent passages of nonadherent cells over Sephadex G10 columns[15] prewashed with warm RPMI-1640 + 20% FCS. Macrophage-depleted cells contained less than 1% neutral red-positive, latex-positive cells. Adherent normal peritoneal cells were obtained by peritoneal lavage and 3-h adherence to plastic Petri dishes. Adherent cells removed in 1:5,000 EDTA–phosphate-buffered saline with a rubber policeman were 70% neutral red-positive, latex-positive. Adherent cells were irradiated with 1,200 rads. 5×10^3 Macrophages were added to cultures of 5×10^5 macrophage-depleted cells. A peak stimulatory dose of 2.5 μg goat anti-IgM, a dose of 0.5 μg *Escherichia coli* K235 LPS, a dose of 0.25 μg Con A or medium only was added to each culture. ^{3}H-thymidine incorporation is expressed as Δ = (experimental minus control) and as stimulation index = (experimental/control). In expt 2, normal spleen cells were incubated with 2.5 μg goat anti-IgM, 0.5 μg K235 LPS, 0.25 μg Con A, or medium. 50 μg silica (particle diameter <5 μm) was added to one series of cultures.

purified γ,κ (MOPC-21) myeloma protein failed to affect the response[13]. Hence, the proliferative response induced by the affinity-purified antibody was apparently due to anti-μ activity and not to any anti-λ or anti-idiotype activity in the preparation.

The spleen cell proliferation induced by anti-IgM seems to be a T-independent phenomenon. Spleen cells from nude mice and spleen cells depleted of T cells by T cell-specific cytotoxic antisera + complement show intact anti-IgM-induced proliferative responses[14] (our unpublished results).

We investigated the requirement for accessory macrophages in this B-cell activation system. Adherent cells were removed from preparations of nucleated spleen cells by 2-h adherence to plastic and two sequential passages through Sephadex G10 columns. Such depleted cells were poor responders to goat anti-IgM (Table 1, expt 1). The addition of irradiated adherent cells from normal peritoneum restored the B-cell response to that seen with normal spleen cell suspensions.

Fig. 1 Stimulation of CBA/Tufts spleen cells with goat anti-IgM. Various concentrations of MOPC-104E column-purified goat anti-(MOPC-104E, TEPC-183 (●) or normal goat Ig (○) were added to 5×10^5 spleen cells in triplicate flat-bottomed microculture wells to give a total volume in each well of 200 μl. Cultures were maintained in RPMI-1640 + 5% heat-inactivated FCS, with 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, 20 mM HEPES (Sigma) and 2 mM glutamine. After 48 h of culture at 37 °C in a humidified, 5% CO$_2$ incubator, 1 μCi of [methyl-^{3}H]thymidine (6.7 Ci mmol^{-1}, NEN) was added to each culture. After 24 h, cells were collected and the ^{3}H-thymidine incorporation was measured. The means of triplicate cultures ± standard deviations were calculated for experimental and control cultures.



Further evidence that macrophages are ancillary cells for anti-IgM-induced proliferation was obtained from experiments in which silica, a selective toxic agent for phagocytic cells[16], was added to cultures. Silica, 50 μg, significantly depressed the spleen cell response to anti-IgM without affecting the response to LPS, another polyclonal B-cell activator (Table 1, expt 2).

The response to LPS was also unaffected by adherent cell depletion (Table 1, expt 1). In fact, the addition of adherent peritoneal cells to depleted cell cultures suppressed the LPS response. These characteristics of B-cell LPS responses have been previously noted[17,18].

Unlike the response to LPS, the B-cell proliferative response to the mitogen dextran sulphate has been shown to be macrophage-dependent[17]. Whether the apparent macrophage independence of the LPS response, in contrast to the dextran sulphate and anti-IgM responses, is a consequence of the unique activating properties of the LPS molecule[19,20] or a reflection of subsets of B cells with different macrophage requirements for activation is not yet known.

Our macrophage depletion techniques failed to decrease the macrophage-dependent T-cell response to concanavalin A (Con A)[21] to the same extent as the B-cell response to anti-IgM (Table 1). This differential sensitivity to macrophage depletion may reflect differences in the amount of macrophage help required for the stimulation of diverse lymphocyte populations or may reflect a heterogeneity in ancillary macrophage populations[22,23].

Our results suggest that, in the presence of macrophages, binding of molecules to surface IgM can activate certain B cells to proliferate. The precise role of the macrophage in this system is not yet clear. Macrophages may contribute secondary signals needed to push B cells further into the cell cycle. Alternatively, they may promote the viability of the B-cell population stimulated by anti-IgM, or they may present anti-IgM to B cells in an insoluble array. We feel the latter possibility is unlikely because: (1) anti-IgM-pulsed and washed macrophages are unable to stimulate macrophage-depleted lymphocytes in the absence of re-added soluble anti-IgM (unpublished); (2) Sieckmann *et al.* have shown that Fab$_2$, which is incapable of Fc-receptor binding to macrophages[24], and whole Ig preparations of goat anti-IgM are equally stimulatory for mouse cells[10]; and (3) 2-mercaptoethanol added to cultures can overcome the effects of macrophage depletion[13]. Whether 2-mercaptoethanol potentiates the effect of residual macrophages in adherent cell-depleted cultures or substitutes for macrophage function in the stimulation of anti-IgM responsive B cells is not known.

The population of B cells which responds to anti-IgM is thus far analogous to the population which responds to most thymus-independent antigens and the population which produces colonies in *in vitro* soft agar cultures. All these functional populations have been found to be reduced or absent in CBA/N mice and to require macrophages, but not T cells, for their activation[25-28].

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Occurrence of Ia antigens on tissues of non-lymphoid origin

UNLIKE the classical transplantation antigens, called HLA-A,B,C in man and H-2K,D in mouse, the HLA-DR (Ia) antigens have a restricted tissue distribution[1]. Primarily cells belonging to the immune system express Ia antigens whose only known biological role is to participate in cell-to-cell cooperation events involving lymphocytes and macrophages[2-4]. However, cells of the immune system do not only interact among themselves but they also associate physically with other types of cells like epithelial cells of the intestine and the lactating mammary gland[5] and with reticuloepithelial cells of the thymus[6]. The possibility that all such interactions are under Ia antigen control is raised by indirect immunofluorescence analyses which show that Ia antigens are expressed on a great variety of epithelia. We report here our examination of several cryostat sections of guinea pig tissues for the presence of Ia antigens by indirect immunofluorescence. To obtain staining patterns that were reproducible and significantly different from the controls it proved necessary to use immunosorbent-purified heteroantibodies (see legend to Fig. 1 for description of the antibodies).

The specificity of the isolated antibodies was tested in indirect immunoprecipitations[7] of radioactive cell membrane glycoproteins[8]. SDS–polyacrylamide gel electrophoresis[9] of precipitated molecules derived from guinea pig splenocytes, intestine epithelial cells, salivary gland epithelial cells and Kupffer cells revealed the occurrence of Ia antigen-like proteins[10] on all four cell types (Fig. 1). The high specificity of the antibodies was ascertained by the absence on the gels of material distinct from the Ia-antigen subunits.

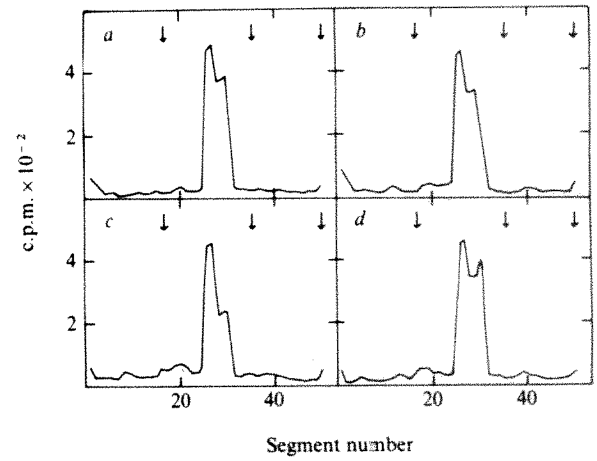

Fig. 1 SDS–polyacrylamide gel electrophoresis of ^{3}H-labelled cell surface glycoproteins indirectly immunoprecipitated with immunosorbent purified antibodies raised against HLA-DR antigens. Single cell suspensions were prepared from spleen (*a*), small intestine[20] (*b*), and parotid gland[21] (*c*), and Kupffer cells were isolated from the liver[22] (*d*) of outbred guinea pigs. The cells were surface labelled with ^{3}H (ref. 8), membrane proteins were solubilised with Triton X-100, glycoproteins were isolated by lectin affinity chromatography and indirect immunoprecipitations were carried out[7]. Control precipitates were generated with normal rabbit serum. Precipitated molecules were subjected to SDS–polyacrylamide gel electrophoresis. The control precipitates did not contain significant amounts of radioactivity (not shown). The immunosorbent-purified antibodies were derived from a rabbit antiserum against highly purified HLA-DR antigens. The reactivity of this antiserum has been documented in detail elsewhere[14] and it has been shown that this antiserum crossreacts extensively with guinea pig Ia antigens[23]. The antiserum was passed over a Sepharose-4B column containing highly purified, covalently bound HLA-DR antigens isolated from chronic lymphatic leukaemia cells (L.K., L.T., L.R. and P.A.P., submitted). The bound antibodies were desorbed with 0.2 M sodium citrate buffer, *p*H 2.9. After extensive dialysis against phosphate-buffered saline (PBS), *p*H 7.2, the antibodies were concentrated. The arrows (left to right) denote the migration positions of marker IgG heavy and light chains and the tracking dye bromphenolblue.

The results of the indirect immunofluorescence studies with use of the specific antibodies on cryostat sections from various tissues are summarised in Table 1. In all tissues examined only epithelial cells were stained. The exception to this was the liver where only the Kupffer cells reacted convincingly with the antibodies whereas the hepatocytes appeared devoid of Ia antigens. The earlier reports that the Langerhan's cells of epidermis express Ia antigens[11,12] were confirmed. The cornea contains similar types of cells as the antibodies bound specifically to cells displaying short dendritic processes. A strong, patchy fluorescence was noted along the border of all intestinal sections studied (Fig. 2*a*). The cells lining the bronchi in the respiratory tractus were generally stained rather diffusely. The epithelia of the gall bladder and the kidney pelvis (Fig. 2*b*) were well stained in some parts and weakly in others. The urinary bladder epithelium also reacted well with the antibodies inasmuch as a strong fluorescence was observed, particularly in the crypts. The mammary gland from virgin animals contained few if any cells that were stained by the anti-HLA-DR antigen antibodies. However, in sections of lactating mammary glands an intensive fluorescence was noted around the epithelial cells lining the ducts (Fig. 2*c*). These cells produce the milk fat droplets which are enveloped by plasma membrane from the epithelial cells[13]. Figure 2*d* shows that also the membranes of

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the milk fat globules displayed a bright fluorescence after staining with the antibodies.

Antibodies gave a discrete but uniform fluorescence mainly on rather large cells when sections of thymus were examined. The antibodies do not react with thymocytes[14] so it seemed that the stained cells probably belonged to the reticuloepithelium.

The relative amounts of the anti-HLA-DR (Ia) antigen antibodies bound to single cells obtained from the small intestine and spleen were compared. Figure 3 shows that those spleen cells that bound the antibodies contained about threefold more

Table 1 Occurrence of Ia-antigen-like molecules on cells of various organs*

Tissue	Cell type stained†	Presence of Ia antigen subunits‡
Salivary glands (parotid and submandibular glands)	Glandular epithelium	+
Oesophagus (upper part)	Not detected	nt
Small intestine (duodenum; jejunum; ileum)	Epithelial cells	+
Colon	Epithelial cells	nt
Liver	Kupffer cells	+
Gall bladder	Epithelial cells	nt
Cornea	Langerhans-like cells	nt
Epidermis	Langerhans' cells	+
Mammary gland		
(virgin)	Few Ia-antigen expressing cells	nt
(lactating)	Epithelial cells	+
Trachea	Not detected	nt
Bronchi	Epithelial cells	nt
Urinary bladder	Epithelial cells	nt
Kidney pelvis	Epithelial cells	nt

* All analyses were performed on tissues from guinea pigs. Ia-antigen-like molecules were identified with use of immunosorbent-purified anti-HLA-DR antigen antibodies.

† Determined by indirect immunofluorescence on thin cryostat sections.

‡ Determined by indirect immunoprecipitation and SDS–poly-acrylamide gel electrophoresis of ^{3}H-labelled cell surface glycoconjugates.

nt, not tested.

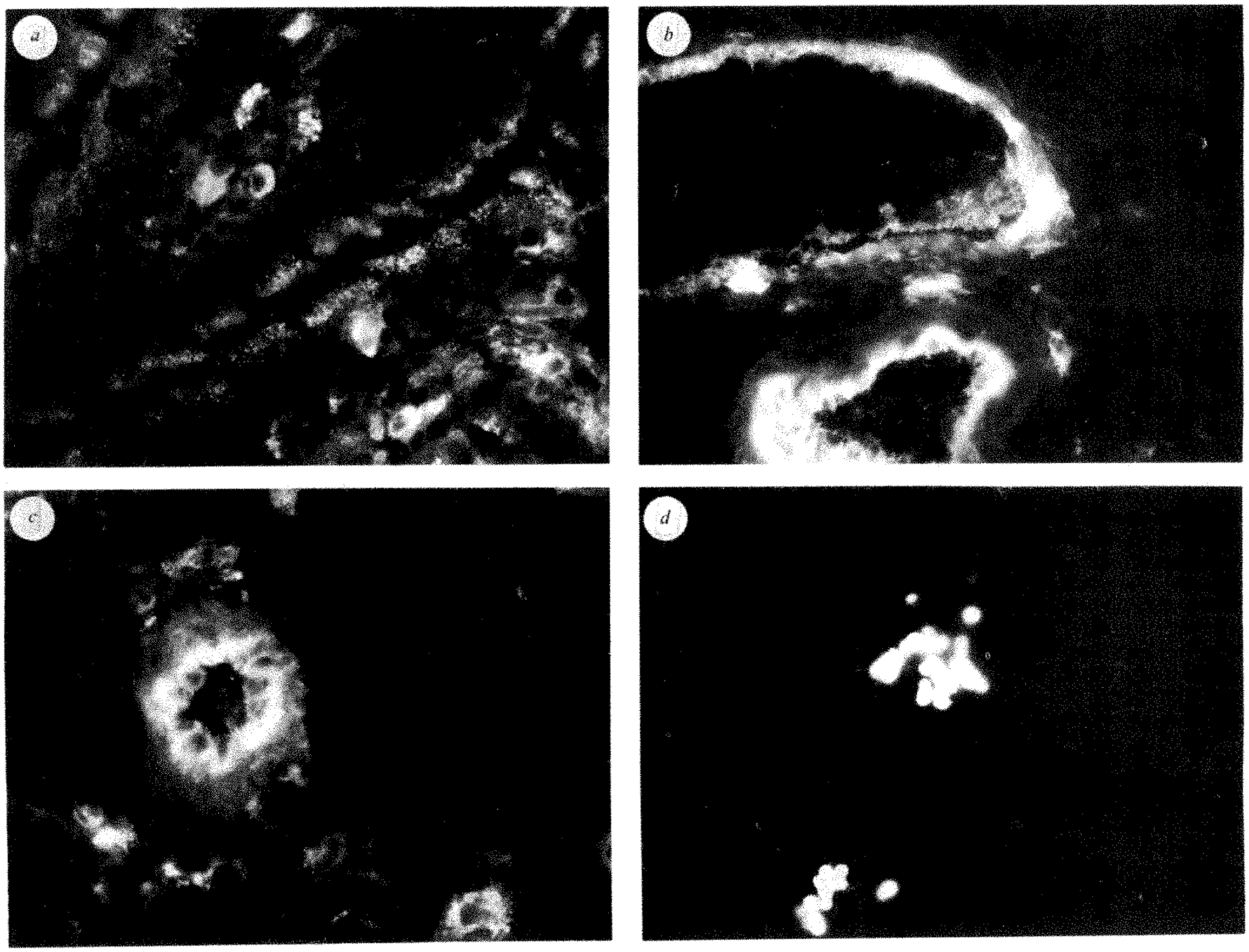

Fig. 2 Indirect immunofluorescence of thin sections of small intestine (*a*), kidney pelvis (*b*), lactating mammary gland (*c*) and milk fat globule membranes (*d*) of guinea pigs after staining with immunosorbent-purified anti-HLA-DR antigens antibodies (see legend to Fig. 1). The tissues were immediately frozen in cold isopenthane (−70 °C) and sections of 6 μm were subsequently prepared. The air-dried sections were incubated with 50-μl portions of the antibodies and normal rabbit IgG, respectively. The IgG-fractions, used at a concentration of about $10\ \mu g\ ml^{-1}$, were diluted in PBS, *p*H 7.2, containing 4% bovine serum albumin (BSA). The antibodies were allowed to react with the sections for 30 min at room temperature in a humidified atmosphere. The sections were then washed extensively in PBS–BSA and allowed to react with fluorescein-isothiocyanate-conjugated swine-anti-rabbit IgG antibodies (Dakopatts) at a concentration of $0.5\ mg\ ml^{-1}$. After an incubation period of 30 min and several washes in PBS–BSA the reactions were mounted in 10% glycerol-PBS, *p*H 7.2. The sections were examined in a Zeiss fluorescence microscope with incident light and blue narrow band activation (chromatic mirror 510 and interference filters KP 490 and KP 500 and orange filter LP 520) illuminated with an Osram HBO 200 lamp. Sections stained with normal rabbit IgG did not display any fluorescence (not shown).

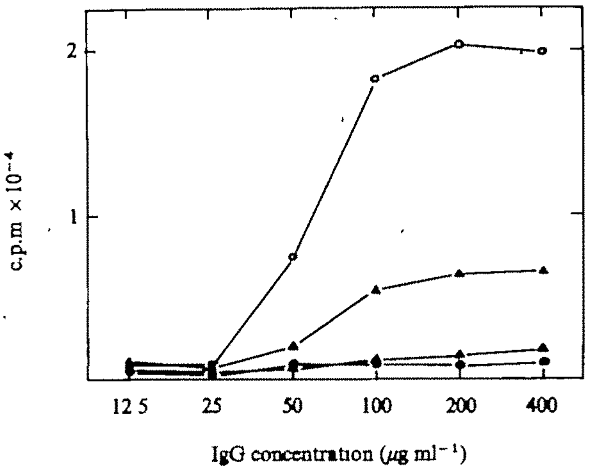

Fig. 3 Binding of immunosorbent-purified anti-HLA-DR antigen antibodies to guinea pig spleen cells (○,●) and to small intestine epithelial cells (△,▲). Aliquots containing 5×10^5 spleen cells and 2×10^5 epithelial cells[20], respectively, were incubated with various amounts of immunosorbent purified antibodies (○,△) and normal rabbit IgG (●,▲), respectively, in a final volume of 0.1 ml of PBS–BSA. After an incubation period of 30 min at +22 °C the cells were washed extensively and suspended in 0.05 ml of PBS–BSA containing saturating amounts of ^{125}I-labelled protein A (ref. 24). After a further incubation period of 30 min at 22 °C the cells were repeatedly washed and subjected to radioactivity measurement. Indirect immunofluorescence of the two single cell suspensions demonstrated that all of the epithelial cells but only about 40% of the spleen cells reacted with the antibodies. The number of spleen cells used in the binding experiment was adjusted accordingly.

of the radioactivity than did the intestine epithelial cells. As the latter type of cells are considerably larger than the lymphocytes the density of the Ia antigens per unit surface area on the epithelial cells must be only a fraction of that of the B lymphocytes.

This study was undertaken with use of immunosorbent-purified heteroantibodies. The occurrence on various tissues of molecules reactive with the antibodies does not necessarily imply that true Ia antigens were recognised inasmuch as the heteroantibodies may have cross-reacted with other components. This possibility seems unlikely, however, as the only cell surface-labelled glycoconjugates that reacted with the antibodies displayed the typical Ia antigen subunit pattern on SDS–polyacrylamide gel electrophoresis. Moreover, highly purified intestinal epithelial cells of mice will give rise to Ia antigen alloantibodies when injected into I-region congenic mice. Such alloantisera react with Ia antigens expressed on splenocytes. Some conventional anti-Ia antigen alloantisera also react specifically with the intestinal cells (B.C., L.R. and P.A.P., unpublished observations). Whether the Ia antigens present on cells of non-lymphoid origin belong to any particular sublocus of the MHC I-region remains to be elucidated.

The demonstration of Ia antigens on epithelial cells in various tissues has bearing upon clinical organ transplantations. The presence of Ia antigens on kidney cells is in keeping with the observation that HLA-DR antigen identity between the donor and the recipient is of as great importance as HLA-A,B,C antigen identity[15].

The association between some types of diseases and the HLA-DR antigen phenotype[16] also has bearing on the present findings. Thus, the expression of HLA-DR antigens of a given phenotype on cells of the disease-prone organs might represent an absolute requirement for manifestation of the disease.

It is interesting that Ia antigens are expressed on reticuloepithelial cells of the thymus in view of the recent observations that the education of T cells in the thymus is dependent on the I-region genotype of the reticuloepithelial cells[17]. It is tempting to suggest that it is the Ia antigens on the reticuloepithelial cells that are crucial for the functional maturation of the T cells. The abundance of IgA-secreting cells in various epithelia[18] may be related to the occurrence of Ia antigens in these tissues. This is particularly important for the mammary gland. In virgin animals very few IgA-secreting plasma cells are found in the gland but under the influence of lactotropic hormones IgA-secreting cells accumulate in the epithelium of the gland[19]. This sequence of events is strictly parallel to the expression of Ia antigens on the epithelial cells (L.K., U.F. and P.A.P., unpublished observations).

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Localisation of Chido and Rodgers determinants to the C4d fragment of human C4

CHIDO (Ch) AND RODGERS (Rg) are blood-group antigens present in plasma and on red cells. Interest in these antigens was stimulated when Ch (ref. 1) and Rg (ref. 2) were found to be genetic markers for the major histocompatibility complex, HLA, and when strong linkage disequilibrium between the Rg-negative phenotype and HLA-A1, B8 homozygosity was noted[3]. Ch and Rg recently attracted a broad audience when O'Neill *et al.*[4] showed that the Ch and Rg phenotype of plasma was correlated with the electrophoretic polymorphism of human C4, the fourth component of complement, and that C4 purified

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Table 1 Agglutinin titres of anti-C4, anti-C3, anti-Ch and anti-Rg with red cells coated with complement

Source of complement	Ch, Rg phenotype	LIS % sucrose	Antiserum					
			Anti-C4c	Anti-C4d	Anti-C3b	Anti-C3d	Anti-Ch	Anti-Rg
EDTA plasma	Ch+, Rg+	10	0	0	0	0	0	0
ACD plasma	Ch+, Rg+	10	>10,000	>1,600	>500	>120	>2,000	>2,000
Serum	Ch+, Rg+	10	128,000	>1,600	>500	>120	2,560	2,560
	Ch+, Rg+	6	64,000				2,560	1,280
Serum	Ch−, Rg+	10	32,000	>1,600	>500	>120	0*	2,560
	Ch−, Rg+	6	500				0	0
Serum	Ch+, Rg−	10	64,000	>1,600	>500	>120	1,280	0*
	Ch+, Rg−	6	100				0	0
C2D serum	Ch+, Rg+	10	>10,000	>200	0	0	>400	>400
C4D serum	Ch−, Rg−	10	0		0	0	0	0
C4D serum	Ch−, Rg−	10	0		0	0	0	0
Serum	Ch+, Rg+	9.24	0	0	>500	120	0	0
	Ch−, Rg+	9.24	0	0	>500	100	0	0
	Ch+, Rg−	9.24	0	0	>500	50	0	0
Anti-Le^{a} serum	Ch+, Rg+	none	>1,000	200			>8	>8

As a source of complement, serum, ACD plasma and EDTA plasma was obtained from donors of known Ch and Rg phenotype. Red cells were coated with complement by activation in low-ionic-strength solution (LIS): 10% aqueous sucrose[8]; 6% sucrose/0.15% NaCl (ref. 12); 9.24% buffered sucrose containing EDTA, purported to coat cells with C3 without detectable C4 (ref. 11). C4D serum was from a C4-deficient patient; C2D serum was from a C2-deficient patient. Immune transfer of complement to red cells was effected using fresh serum containing anti-Le. Red cells were tested with anti-complement reagents diluted in 22% bovine serum albumin (Ortho Diagnostics). Rabbit anti-human C4 (Behringwerke AG) reacted strongly with red cells coated with C4b but not with these cells after trypsin treatment, or with red cells sensitised *in vivo* with C4/C3, this serum is referred to as anti-C4c. Anti-C4d (two examples) was raised in monkeys injected intravenously with autologous red cells coated with C4d only; these sera were non-precipitating[5]. Anti-C3b (Behringwerke) was obtained as anti-human C3. Anti-C4c, anti-C4d, and anti-C3b were used unabsorbed at dilutions of 1 . 50 or greater. Anti-C3d was absorbed with human red cells. Six examples of anti-Ch and five examples of anti-Rg from patients immunised by transfusion or pregnancy were used.

* Weak and variable background reactions were observed at all dilutions of antiserum; these reactions were abolished by trypsin-treatment of the red cells and were probably due to immune adherence

to homogeneity inhibited anti-Ch and anti-Rg. We have studied the transfer of Ch and Rg from plasma to red cells in conditions in which C4 is transferred. We report here a correlation between the Ch, Rg polymorphism and functional C4, and show that Ch and Rg reside on the C4d fragment of the molecule. We also report that a monkey antiserum to human C4d (ref. 5) is predominantly anti-Ch and anti-Rg.

The antibodies anti-Ch and anti-Rg have been described as 'nebulous'[6] and 'serologically difficult'[7] because of their weak and variable reactions with red cells; Ch and Rg can usually be detected only by the antiglobulin test. We found, however, that Ch and Rg could be readily detected by direct agglutination using anti-Ch and anti-Rg when the antigens were transferred to red cells *in vitro* by activation of the classical complement pathway in low-ionic-strength solution (LIS). In 10% sucrose LIS[8], red cells became strongly Ch(Rg)-positive when serum or ACD plasma was used as a source of C4, but not when EDTA plasma was used; the Ch(Rg) phenotype acquired by the red cells was entirely dependent on the Ch(Rg) phenotype of the donor serum (Table 1). Red cells acquired both Ch and Rg from C2-deficient serum, but neither Ch nor Rg from C4-deficient serum; C4-deficient serum did not inhibit anti-Ch or anti-Rg. Heating serum to 56 °C for 60 min (ref. 9), or treatment with thiocyanate[10] which inactivates C4, C3 and C5, abolished the transfer of Ch(Rg) to red cells in LIS. Using a LIS technique[11] which coats red cells with C3b without C4, the cells did not acquire Ch(Rg). Immune transfer of Ch(Rg) was demonstrated by exposing Le(a+) red cells to fresh serum containing complement-binding anti-Le^{a} (Table 1).

We found that Ch+, Rg− and Ch−, Rg+ serum could be readily distinguished from Ch+, Rg+ serum by the amount of C4 transferred to red cells in 6% sucrose/0.15% NaCl LIS[12], but such a distinction could not be made when 10% sucrose LIS was used (Table 1). This finding was unexpected but accords with the known correlation between the immunochemical polymorphism of C4 and functional activity of the molecule[13]. Determination of plasma C4 by single radial immunodiffusion (Behringwerke plates) showed that Ch+, Rg+ plasma usually contained more C4 than Ch+, Rg− plasma did, and that Ch−, Rg+ plasma contained the least C4. However, when Ch+, Rg− or Ch−, Rg+ plasma could be matched for C4 content with Ch+, Rg+ plasma, the amount of C4, Ch, Rg transferred in 6% sucrose/0.15% NaCl LIS was again correlated with the Ch(Rg) phenotype. This result suggests that there are intrinsic differences in the activity of C4 molecules of different Ch, Rg phenotype.

Previous studies assessing the effects of reducing agents and enzymes[14] on incomplete blood-group antibodies suggested that, in agglutination tests, the Chido antigen on normal red cells is not destroyed by trypsin. We investigated further this apparent localisation of Ch(Rg) to a trypsin-resistant fragment of C4. In a kinetic study, red cells coated with C4, Ch, Rg in 10% sucrose LIS lost all reactivity with anti-C4c within about 2 min of exposure to trypsin at 37 °C, whereas C4d and Ch(Rg) determinants still remained at 60 min (Table 2). C4d and Ch(Rg) determinants were resistant to further cleavage, by chymotrypsin, in conditions in which C4c determinants were lost from cells coated with C4b in LIS (Table 2). The reactions of anti-Ch and anti-Rg with cells treated with trypsin, however, became slightly weaker with time indicating some alteration or destruction of Ch(Rg) with prolonged exposure to the enzyme. This finding correlates with the observation that, in the antiglobulin test, Ch and Rg on normal red cells are much weaker after enzyme-treatment. Since agglutination tests confirmed that Ch(Rg) is borne by a tryptic-C4d fragment of C4, we tested red cells expected to carry C3bINA-cleaved C4d due to complement-activation *in vivo*[15,16]. Such cells, collected into EDTA from patients with cold autoimmune haemolytic anaemia, failed to react with anti-C4c but were strongly agglutinated by anti-C4d, and by anti-Ch and anti-Rg (Table 2).

In all experiments anti-Ch(Rg) behaved the same as anti-C4d. As the monkey anti-C4d was non-precipitating[5], it might be directed against few antigenic determinants, possibly just Ch and Rg. In inhibition tests using red cells coated with C4d, Ch, Rg, the anti-C4d was inhibited by Ch+, Rg+ serum but there was no detectable inhibition with Ch−, Rg+ or Ch+, Rg−

serum. Anti-C4d was inhibited by mixing Ch−, Rg+ and Ch+, Rg− serum but the inhibition was less than with Ch+, Rg+ serum. Thus this anti-C4d is predominantly anti-Ch and anti-Rg. This surprising finding presumably reflects the phylogenetic proximity of monkeys to man.

The preservation of Ch and Rg on C4-sensitised red cells exposed to trypsin and chymotrypsin *in vitro*, or C3bINA *in vivo*, indicates that these antigens reside on the limit fragment, C4d, which is stably associated with the cell surface[17]. Ch and Rg must, of course, be spatially distant from that portion of C4 which interacts with the membrane. It is interesting that Ch and Rg survive cleavage and presumed conformational change in C4 which precede transfer of the molecule to the red cell. Conformational changes presumably also accompany processing of single-chain precursor C4 (ref. 18) to the mature three-chain structure; anti-Ch(Rg) could be used as a probe for such changes. As the structural locus for C4 (refs. 19, 20) and *Ch* (ref. 1) and *Rg* (ref. 2) all map to the same small region of chromosome 6, it is likely that primary structural differences, rather than some obligatory post-translational modification such as glycosylation[21], generate the Ch, Rg antigenic polymorphism. It does not follow, however, that the electrophoretic polymorphism of C4 is entirely due to the structural differences in C4d which distinguish Ch from Rg. Certainly, in some gel systems[22,23] the apparent structural variability cannot be explained solely by Ch and Rg differences. The structural polymorphism of C3 is, in fact, in the C3c fragment[24,25], and C4 and C3 are homologous proteins.

Graham *et al.*[26] have shown that C3d is present on normal red cells. To the extent that normal red cells carry detectable Ch and Rg, these cells are also coated with C4d. A cardinal question, therefore, concerns the origin of the natural Ch(Rg) on red cells. Does natural Ch(Rg) represent low-level sensitisation with C4 during physiological activation of the classical complement pathway, or is it bound by a different mechanism? A unified hypothesis that all Ch(Rg) on red cells is dependent on complement activation would accommodate the serological differences between natural Ch(Rg), and Ch(Rg) acquired by known activation of complement. The nebulous and variable reactions of anti-Ch and anti-Rg with normal red cells are presumably due to only trace amounts of C4d on the cells.

Table 2 Effect of enzymes on C4, Ch(Rg) bound to human red cells

Method of coating red cells with complement	Enzyme treatment of C4-coated red cells: Enzyme	Min	Antiserum: Anti-C4c	Anti-C4d	Anti-Ch	Anti-Rg
LIS	None		>50,000*	>1,600	3,200	3,200
in vitro	Trypsin	2	100	>1,600	3,200	3,200
	Trypsin	5	0	>1,600	3,200	3,200
	Trypsin	10	0	>1,600	3,200	3,200
	Trypsin	30	0	>1,600	1,600	1,600
	Trypsin	60†	0	>1,600	1,600	1,600
	Chymotrypsin	30	200	>1,600	3,200	1,600
	Trypsin Chymotrypsin	30 30	0	>1,600	3,200	3,200
Auto-anti-I	None		0	>400	>8	>16
in vivo	Trypsin	30	0	>400	>8	>16
Auto-anti-P	None		0	>400	>8	0
in vivo	Trypsin	30	0	>400	>8	0

One volume of packed red cells, coated with C4, Ch, Rg in 10% sucrose LIS containing Ch+, Rg+ serum, was incubated at 37 °C in four volumes of 1 mg ml^{-1} Trypsin-TPCK (Worthington Biochemical) in 20 mM Tris-HCl/0.15 M NaCl, pH 7.8 (TBS). At intervals, an aliquot of red cells was removed, centrifuged through a large volume of saline, and washed a further three times. After 30 min, a portion of these cells and cells freshly coated with C4, Ch, Rg were incubated with α-chymotrypsin (Worthington), 1 mg ml^{-1} TBS containing 10 mM $CaCl_2$ for 30 min at 37 °C, then washed three times. Red cells coated *in vivo* with C4/C3 were obtained from patients with cold autoimmune haemolytic anaemia In two Ch+, Rg+ patients the auto-antibody had I specificity, in one patient, who was Ch+, Rg−, the auto-antibody had P specificity. The anti-complement reagents used are described in the footnote to Table 1.

* Haemagglutinin titre. † One volume fresh trypsin added at 30 min

As a number of complement components exhibit polymorphism, there is a potential for formation of alloantibodies to these components by transfusion. However, these components may have to be cell-bound to elicit an immune response. Anti-Ch and anti-Rg, elicited by C4, may be the first examples of alloimmunoconglutinins in man.

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Effective subunit vaccines against an enveloped animal virus

VACCINES are available to act against many of the diseases caused by enveloped viruses. The efficacy and safety of the vaccines depend on the method used to convert the virus into a vaccine. Whole viruses that have been attenuated or killed are effective, but both of these types of vaccines may give rise to undesirable side effects which limit their use[1–3]. These complications are caused by the virus genome or by toxic components in the virus preparations. The components that give rise to the side effects do not seem to be those that are important for evoking protective immunity and it is therefore possible to prepare vaccines which contain only the necessary antigenic subunits, which for the enveloped viruses are the spike glycoproteins[4–7]. But although subunit vaccines have proved safer

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Table 1 Chemical composition of subunit vaccines used

Vaccine preparation	Polypeptide composition	Molecular weight	No. of virus spikes per complex	Lipid content (mg per mg protein)	Detergent content (mg per mg protein)	Infectivity* (PFU)	Ref.
4.5S complexes	E1, E2, E3	1.5×10^5	1	0.01	0.54	NT	16
29S complexes	E1, E2, E3	9.5×10^5	8	0.01	0.03	<10	17
Virosomes	E1, E2, E3	—	~20–2,000†	0.9–1.0	0.05	<10	15

NT, not tested (detergent interferes with assay).

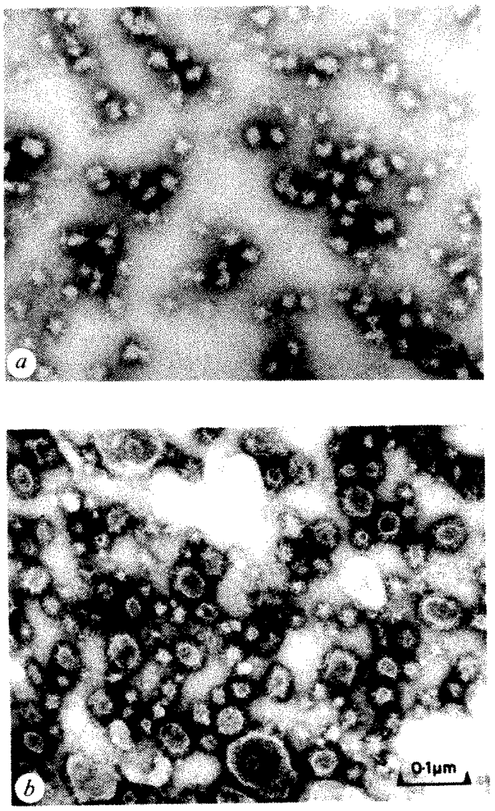
* Infectivity assayed by plaque titration in BHK-21 cells[33] at a protein concentration of 0.2 mg per ml.

† The virosomes consist of a heterogeneous population of vesicles of different sizes.

than whole virus vaccines, their immunogenicity is generally lower[8,9], perhaps because the form in which the proteins are presented in the vaccines is not optimal for eliciting a strong immune response[10]. In support of that explanation, we present here evidence that a lethal infection of mice with Semliki Forest virus can be prevented by a single vaccination with the virus spike protein, provided it is presented as a micellar aggregate of the protein or as protein reconstituted into vesicles of egg lecithin. The monomer form of the spike protein solubilised with detergent is very much less effective.

Semliki Forest virus (SFV) is well suited for the studies outlined above[11,12]. It is a simple enveloped virus in the Togaviridae family. In mice lethal strains cause encephalomyelitis which leads to death within a week. The surface glycoproteins of this virus have been studied in detail[13]. Systematic studies of the solubilisation[14] and reconstitution[15] of the virus membrane have yielded methods for the preparation of three different physical forms of the spike protein; (1) the 4.5S complex[16], a complex of one spike protein bound to 75 molecules of Triton X-100; (2) the 29S complex[17], a protein micelle of eight spikes virtually devoid of lipid and detergent and with a molecular weight of 9.4×10^5; and (3) virosomes (see ref. 18 for terminology) in which the spike proteins have been reconstituted into vesicles of egg lecithin[15].

Fig. 1 Electron micrographs of *a*, the 29S complexes and *b*, the virosomes used for vaccination. The preparations have been negatively stained.



The vaccine preparations for these studies were made from the prototype strain of SFV which has been passaged in BHK-21 cells several times in our laboratories and is avirulent for mice. Monomers of spike proteins were isolated as 4.5S complexes from the purified virus by disrupting the virus with Triton X-100 and then subjecting this mixture to sucrose gradient centrifugation in the presence of a detergent[16]. The proteins obtained are virtually lipid-free and soluble by virtue of the bound detergent. The 29S complexes were prepared by centrifugation of virus disrupted with Triton X-100 into a sucrose gradient, which separates the lipid, detergent and nucleocapsids from the spike proteins[17]. These aggregate to octameric complexes which are water soluble (Fig. 1*a*). They are similar in structure to detergent micelles. The protein micelles have a polar surface and the hydrophobic tails of the spike proteins are buried in the interior[19]. Other virus spike proteins also aggregate to form soluble complexes (see ref. 19). The virosomes were prepared by the octylglucoside dialysis method[15]. Briefly, the virus was solubilised with Triton X-100 and the spike glycoproteins isolated by sedimentation into a sucrose gradient containing the nonionic detergent β-D-octylglucoside. Egg lecithin solubilised with octylglucoside was added (the lipid/protein ratio being 1 : 1, w/w) and the detergent removed by dialysis (Fig. 1*b*). The properties of the three vaccines are summarised in Table 1.

The amount of 29S complex required to induce protective immunity after a single inoculation into BALB/c mice was studied first. A virus does of 10^4 plaque forming units (PFU) of the lethal L10 strain[11] was used to challenge the mice two weeks after vaccination. The response to increased doses of the vaccine was linear; 0.5 μg of 29S vaccine was sufficient to protect 50% of the mice. A dose of 10 μg induced protection in all animals. Therefore, 10 μg of virus protein was subsequently used in all experiments. We carried out three series of experiments using the three forms of vaccines (Table 2). In the first, the challenge

Table 2 Vaccination against SFV encephalomyelitis in BALB/c mice: the protective effect induced by different forms of purified spike protein vaccine

Physical form of vaccine	Dose (μg protein)	LD_{50} of challenge virus ($\log_{10}$ of PFU) Expt 1	Expt 2	Expt 3
(control)		2.3	2.1	2.6
4.5S complex (monomer)	10	ND	2.7	3.4
29S complex (octamer)	10	>3.5	>5.3	>6.3
Virosome (multimer)	10	>4.0	>5.1	>6.6

The vaccination experiments were performed on 5–6-week-old female BALB/c mice. The mice were vaccinated with a single dose of 10 μg spike protein in 100 μl Eagle's minimal essential medium. One third of the dose was injected subcutaneously and two thirds intraperitoneally (i.p.). Two weeks later groups of 10 mice were challenged i.p. with virulent strain L10.H6.C1 SFV with doses ranging from 50 to 10^4 PFU (Expt 1) 50 to 10^5 PFU (Expt 2) and 50 to 10^7 PFU (Expt 3). The infectivity was determined by plaque titration in BHK-21 cells[33]. The amount of protein in the vaccine preparations was measured by the method of Lowry *et al.*[34] using 0.1% SDS in the reaction mixture. The LD_{50} was calculated as described by Spearman and Kärber[35]. ND, Not determined.

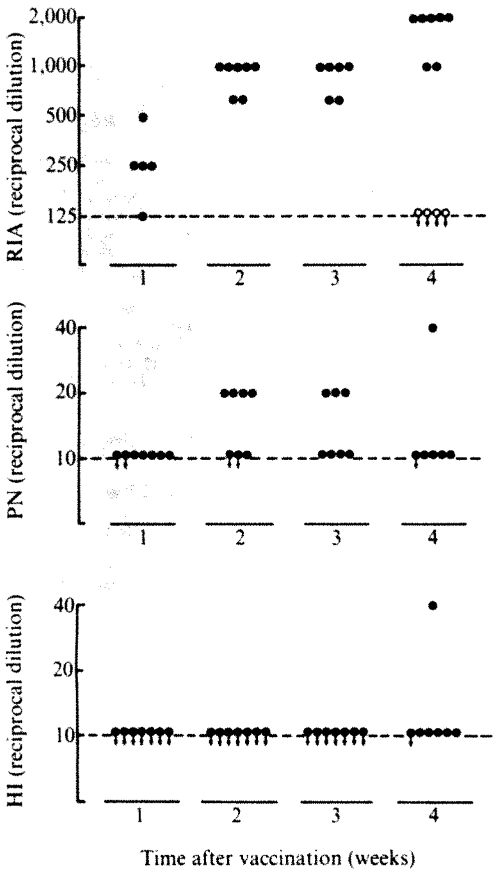


Fig. 2 The antibody response in serum of normal BALB/c and BALB/c nude (*nu/nu*) mice following vaccination with 10 μg SFV spike protein in virosomes. The sera from normal mice were collected weekly 1–4 weeks after vaccination. The nude mice were challenged with 10^3 PFU virulent virus two weeks after vaccination and after a further two weeks the sera from survivors were collected. The radioimmunoassay (RIA) was carried out in a solid phase system using flexible microtitre plates with U-formed bottom (Cooke no. 22–24). The wells were filled with 1 μg virus in 80 μl phosphate-buffered saline (PBS) and incubated overnight at 4 °C. Thereafter the wells were rinsed 3 times with 100 μl PBS, 30% fetal calf serum and 100 μl of mouse serum to be assayed was added in different dilutions and incubated for 1 h at room temperature. After washing 100 μl of immunosorbent purified ^{125}I anti-mouse γ globulin was added and incubated for 1 h at room temperature. After washing, the wells were cut out and radioactivity determined in a gamma counter (Nuclear Chicago). All sera were tested in triplicate and sera from non-immunised were included as negative controls. The titre was defined as the highest dilution of serum which bound more than 2 times the amount of radioactivity of normal mouse serum controls. The plaque neutralisation (PN) test and the haemagglutination inhibition (HI) test were performed as described previously[32,36]. ●, Titres from individual normal BALB/c mice; ○, titres from individual nude mice. The arrow indicates that the titre was below the dilution tested. ---, threshold level of the test, that is, the lowest dilution tested.

dose ranged from 50 to 10^4 PFU and in the second and third series the maximum challenge dose was increased to 10^5 and 10^7 PFU, respectively. Even with the highest challenge dose (10^7 PFU) most of the mice vaccinated with the 29S complexes and the virosomes survived. The extent of protection measured by the difference between the LD_{50} in controls and in the groups vaccinated with a single dose of 29S complexes and of virosomes must thus be higher than $10^{3.7}$ (Table 2). We also vaccinated a group of 10 mice twice with virosomes (10 μg spike protein) two weeks apart and challenged them 14 days later. All the mice survived the highest dose of virus (10^7 PFU).

Mice vaccinated with the 4.5S complex were only slightly protected (Table 2). To verify the very small difference induced by the 4.5S complex, the non-vaccinated mice and the mice vaccinated with the 4.5S complexes that survived the first challenge dose of virus were given a second challenge of 10^4 PFU of virus (Table 3). All but one of the non-vaccinated mice died, whereas the survival of the vaccinated mice was dependent on the dose of virus given in the first challenge. If the first challenge was higher than one LD_{50} dose ($>10^2$ PFU), the mice survived the second challenge. Thus vaccination with the monomeric form of the spike protein gave a small but detectable protection against the disease.

Table 3 Enhancement by live virus challenge of a poor protective effect induced by vaccination with 4.5S complexes

Vaccine* (day 0)	1° Challenge (day 14) Dose (PFU)	Mortality†	2° Challenge (day 28) Dose (PFU)	Mortality	Comments
None	$10^{1.5}$	0/10	10^4	10/10	No protection obtained after 1st challenge
	$10^{2.0}$	1/10	10^4	9/9	
	$10^{2.5}$	5/10	10^4	4/5	
	$10^{3.0}$	24/30	10^4	6/6	
4.5 complex (10 μg protein)	$10^{1.5}$	0/10	10^4	8/10	First challenge with a dose $<1\ LD_{50}$
	$10^{2.0}$	3/10	10^4	5/7	
	$10^{3.0}$	4/10	10^4	0/6	First challenge with a dose $>1\ LD_{50}$
	$10^{4.0}$	8/10	10^4	0/2	

* Vaccination was performed as described in Table 2. First challenge was performed 2 weeks after vaccination, and a second challenge after a further 2 weeks. For details see Table 2.

† Mortality expressed as number of mice killed per total number of group.

The dependence on T cells for induction of protective immunity using virosomes was studied by comparing the response of BALB/c mice with that of BALB/c nude (*nu/nu*) mice (Table 4). The LD_{50} for non-immunised nude mice was higher than for normal BALB/c. In contrast to normal mice no difference in survival time or rate between non-vaccinated and vaccinated mice was observed in *nu/nu* animals. The total serum antibody response was measured with a radioimmunoassay. No antibodies were detected in vaccinated nude mice which were challenged after two weeks and tested after four weeks. In normal mice the serum antibody titres kept rising from the first to the fourth week after immunisation. Antibody against SFV was detectable by plaque neutralisation assays 2 weeks after vaccination, and antibody causing haemagglutination inhibition after four weeks. The titres were low (Fig. 2). The above results suggest that T cells are required for protective immunity, as has been found for several viruses[20]. More detailed immunological studies are underway to explore the mechanisms of protection against SFV infection.

Previous studies have also shown that solubilisation of togaviruses with different mild detergents leads to vaccines with low efficacy[12]. Monomeric forms of spike proteins have been isolated from other enveloped viruses, and they too are poor immunogens. In contrast to SFV spike protein the Friend leukaemia virus spike glycoprotein (gp71) is a typical peripheral membrane protein which does not need detergent for solubility and seems to be attached to the viral membrane through another integral membrane protein[21]. The mice were only partially protected against leukaemia after vaccination with two doses of 100 μg of spike protein in Freund's adjuvant[5,21]. A monomer preparation of the influenza virus haemagglutinin has been prepared by releasing the hydrophilic portion of the spike using a protease[22]. This monomer haemagglutinin was only weakly immunogenic in hamsters and man[23,24]. These results agree with the known poor immunogenicity of monomeric compared with multimeric forms of proteins[25–27]. Monomers of spike protein, water soluble with or without detergent, are clearly not suitable as subunit vaccines. One reason why the virosomes and the protein micelles proved so efficient as vaccines is probably due to the multimeric structure of the spike proteins. The virosomes

Table 4 Resistance of vaccinated and non-vaccinated nude and normal BALB/c mice to infection with a lethal strain of SFV

	Nude			Normal		
Vaccine	Challenge dose (PFU)	No of mice killed per group	Survival time (d)	Challenge dose (PFU)	No of mice killed per group	Survival time (d)
Virosome	10^4	8/9	9.3±5.9	10^4	0/10	—
None	10^4	8/10	13.0±5.5	10^4	10/10	4.4±0.52
Virosome	10^3	1/5	7	10^3	0/10	—
None	10^3	0/5	—	10^3	9/10	5.11±0.93
Virosome	$10^{2.5}$	1/5	6	$10^{2.5}$	0/10	—
None	$10^{2.5}$	2/5	6	$10^{2.5}$	10/10	5.3±1.06

Mice were vaccinated with virosomes containing 10 μg spike protein and challenged 14 d later with different doses of virus. For details see Table 2.

contain a high surface density of spike protein similar to the surface of the virus itself. The 29S complexes contain eight spike proteins held together by their hydrophobic ends. Both forms bind effectively to the surface of cells apparently in such a way that spike proteins remain available for interaction with cells of the immune system[28]. We have recently shown that 29S complexes bind to virus receptors on the cell surface and do not seem to integrate into the cell membrane[29]. These findings suggest that integration of viral antigens into host cell surface membranes may not be a prerequisite for induction of protective immunity.

Another reason for the effectiveness of these vaccines is probably that mild methods were used to extract the spike proteins from the virus and to prepare the multimeric vaccines. Triton X-100 and octylglucoside are non-ionic detergents known to preserve the biological activities of membrane proteins[30,31]. Organic solvents and other denaturing agents such as ionic detergents may destroy antigenic determinants on the spike proteins. Whether protein micelles or virosomes will prove efficient as vaccines for other enveloped viruses has yet to be investigated.

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Fibronectin binds to *Staphylococcus aureus*

MANY important activities have been ascribed to fibronectin, which is found in soluble form in many biological fluids and in insoluble form on the surface of fibroblasts[1–3]. Fibronectin mediates the attachment of fibroblasts and hepatocytes to culture dishes[4–6] and, when solubilised from the fibroblast surface, it binds to the surface of cultured transformed fibroblasts, restoring their morphology to normal[7,8]. I now report that the affinity of fibronectin to cell surfaces extends to the prokaryotic cell *Staphylococcus aureus*.

When labelled fibronectin was incubated with both commercial strains and clinical isolates of *S. aureus* up to 70% of the radioactivity bound to the bacteria. *Mycobacterium butyricum*, however, showed only slight binding (Fig. 1). No ^{125}I-α-feto protein bound to staphylococci nor did fibronectin bind to formalinised sheep red blood cells.

Table 1 Enrichment of fibronectin with *Staphylococcus aureus*

	Fibronectin concentrations in supernatants and eluates (μg ml^{-1})			
	Expt 1		Expt 2	
	Supernatant	Eluate	Supernatant	Eluate
S. aureus	0.1	11.5	0.3	12.5
M. butyricum	6.2	0.6	4.6	0.8
Control*	ND	ND	2.9	0.7

Citrated human plasma, 10–20 μl, containing 200–500 μg ml^{-1} fibronectin was diluted to 0.5 ml with phosphate buffered saline (PBS) and incubated with 0.5 ml 10% suspension of bacteria overnight. Supernatants were collected after centrifugation at 1,000 *g* for 30 min, and the bacteria were washed two times with 2.0 ml PBS. Fibronectin bound to bacteria was eluted adding 150 μl 8 M urea containing 0.05 M Tris, *p*H 8.0, and fibronectin was quantitated by radioimmunoassay. ND, not determined.

* Control tubes were processed as above without any bacteria.

Staphylococcal binding was confirmed using unlabelled fibronectin. After incubation of normal human plasma with *S. aureus* and *M. butyricum*, radioimmunoassay[9] showed marked enrichment of fibronectin in eluates of staphylococci but not in eluates of mycobacteria or in control eluates (Table 1).

To characterise the binding, various substances were tested for the capacity to inhibit binding of ^{125}I-fibronectin to staphylococci. As expected, unlabelled fibronectin was inhibitory, giving half maximal inhibition at 3–4 μg per ml of purified fibronectin. Inhibition by plasma samples containing known amounts of fibronectin was compatible with inhibition by purified fibronectin in the absence of plasma components (Fig. 2). Of the sugars tested, only nonacetylated amino sugars were inhibitory. Half maximal inhibition was obtained with 0.5 M solutions (Table 2). L-Lysine and urea were also inhibitory, whereas divalent cations or chelating agents had no effect on binding. Denatured collagen showed a maximal binding of only 18%, which was not concentration-dependent between 100 μg ml^{-1} and 5 mg ml^{-1}. This slight inhibition might be due to steric inhibition by collagen molecules bound to fibronectin. Protein A, which is present in the cell wall of *Staphylococcus aureus* and binds to immunoglobulins, did not inhibit at 100 μg ml^{-1}. Also F(ab')$_2$ fragments of purified anti-fibronectin antibodies failed to inhibit staphylococcal binding but inhibited the binding of fibronectin to collagen-Sepharose (data not shown). F(ab')$_2$ fragments were used rather than whole antibodies to avoid the binding between protein A and Fc fragments of antibodies.

Thus the binding of fibronectin to *S. aureus* resembles the binding of cells to collagen-coated culture dishes, a process in which fibronectin has been shown to mediate attachment[4,5,10]. Like staphylococcal binding, attachment to culture dishes is inhibited by amino sugars and lysine, although divalent cations are not required for the process. The lack of inhibition of staphylococcal binding in the presence of denatured collagen shows that this binding is not related to the binding of fibronectin to collagen[11].

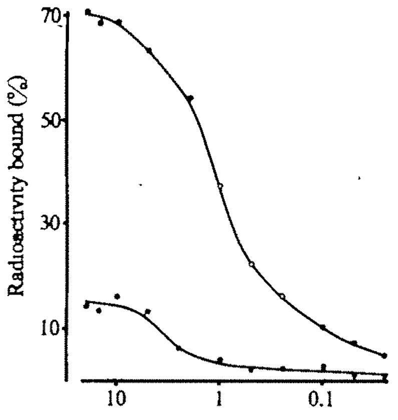

Fig. 1 Binding of ^{125}I-human fibronectin to fixed *S. aureus* (○) and *M. butyricum* (●). Fibronectin was isolated from normal citrated human plasma using affinity chromatography on denatured collagens[11] and radioiodinated using the chloramine-T method[9]. The labelled fibronectin showed a sharp peak of radioactivity co-migrating with the unlabelled fibronectin (MW 220,000) in SDS–polyacrylamide gel electrophoresis in the presence of β-mercaptoethanol[12]. Commercially available heat-killed and formalin-fixed *S. aureus* (Pansorbin, Calbiochem) and *M. butyricum* (Difco) were used. *S. aureus* isolated from routine clinical specimens, heat-killed and fixed with 10% trichloroacetic acid was also used in some experiments, with the same results. The bacteria were washed once with 5 volumes of 4.0 M urea solution containing 0.05 M Tris, pH 7.5, twice with 15–20 times the volume of buffer without urea, and used as a 10% suspension (v/v) in PBS containing 0.1% bovine serum albumin (BSA). Decreasing amounts of 10% bacterial suspension were adjusted to 1.0 ml with PBS–BSA, and ^{125}I-human fibronectin (1.0–1.5 × 10^4 c.p.m.) was added in 50 μl. After incubation overnight at room temperature under continuous rotation, the bacteria were isolated by centrifugation (1,000*g* for 30 min), washed once with 1.0 ml PBS–BSA, and transferred in 250 μl of PBS–BSA into new tubes for counting. Each point represents the mean of duplicate tubes.

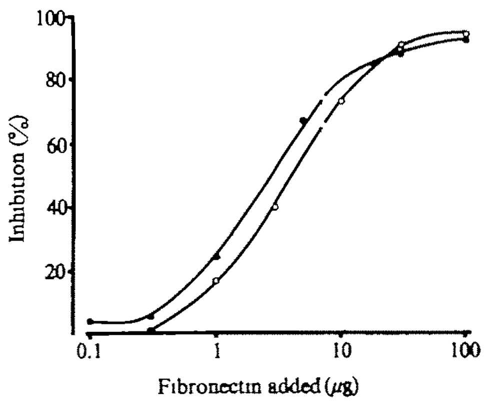

Fig. 2 Inhibition of binding of ^{125}I-human fibronectin to *S. aureus* with normal human plasma (●) and fibronectin isolated from human plasma (○). Increasing amounts of normal human plasma, containing 210 μg ml^{-1} fibronectin, and isolated human plasma fibronectin were incubated with 50 μl of a 10% bacterial suspension. This amount of suspension gave about 50% of maximal binding. The test was carried out as for Fig. 1. Inhibition percentages were calculated according to the formula:

$$\frac{[\text{Binding \% without inhibitor} - \text{binding \% with inhibitor}]}{\text{Binding \% without inhibitor}} \times 100.$$

This conclusion is further supported by the observation that F(ab')$_2$ fragments of anti-fibronectin antibodies do not inhibit staphylococcal binding but inhibit and reverse the binding of fibronectin to collagen[9]. Whether fibronectin also binds to living *S. aureus* and whether it is the anti-staphylococcal factor remains to be seen. If staphylococcal binding is an expression of the affinity of fibronectin for cell surfaces, it could prove useful in the elucidation of the nature of the cellular receptor(s) involved.

Table 2 Inhibition of binding of labelled human fibronectin to *Staphylococcus aureus*

Inhibitor	Concentration needed to inhibit binding (mM) 20%	50%
D-Glucosamine[a]	150	500
D-Galactosamine[a]	ND	500
L-Lysine[b]	100	>1,000
NaCl	100	>2,000
Urea	600	1,050
Anti-human fibronectin F(ab')$_2$ (μg ml^{-1})	>190	—

Binding assay and calculations for inhibition percentages were as described in Figs 1 and 2. The solutions were neutralised with NaOH (a) or with HCl (b) before incubation with bacteria. Amino sugars were used in HCl form, and they were neutralised to pH 7.5 with NaOH before testing. Conductivity measurements showed that 0.5 M stock solutions of these sugars contained the equivalent of 0.2 M NaCl above the conductivity of PBS–bovine serum albumin–buffer alone. This amount of NaCl inhibited the binding 28%. F(ab')$_2$ fragments of purified rabbit anti-human fibronectin antibodies were obtained as follows: 2.4 mg purified antibodies were incubated with 500 μg pepsin in 0.1 M sodium acetate buffer, pH 4.5, overnight at room temperature. Digestion was stopped by dialysis against 0.05 Tris-HCl buffer, pH 8.0, and the digest was passed through a Sepharose–protein A column. Protein peak consisting of unbound material was collected and used in the tests. No inhibition was obtained with the following substances. 1 mM MgCl$_2$ and CaCl$_2$, 100 mM EDTA and EGTA, 0.5 M D-glucose, D-galactose, D-*N*-acetylglucosamine, D-*N*-acetylgalactosamine, L- and D-fucose, D-mannose, L-rhamnose, L- and D-xylose, 0.25 M D-*N*-acetylneuraminic acid and 1 M glycine. ND, not determined.

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The amino acid sequence of *Physarum* actin

THE realisation that actin is involved not only in contractility in muscle tissue but also in general cytoplasmic movement and cell locomotion was based on the isolation of actin from lower eukaryotic organisms, such as *Physarum polycephalum*[1], which show active protoplasmic streaming. It is now accepted that actin is the major structural protein of microfilaments characteristically found in the cytoplasm of most if not all higher cells, and that actins have been highly conserved during eukaryotic evolution and differentiation due to the constraints imposed by the large number of specific interactions which actin shows with other cytoplasmic proteins (for a review see ref. 2). Preliminary fingerprint analysis on *Physarum* actin[3] and partial amino acid sequence analysis on actin from *Acanthamoeba* (ref. 4 and Elzinga cited in ref. 2) have emphasised this point, although so far a complete amino acid sequence has not been reported for actin from a lower eukaryotic species. Our studies on the primary structure of different mammalian actins have shown that several cytoplasmic actins are very similar if not identical in amino acid sequence, although they differ by at least 25 amino acids from the corresponding skeletal muscle actin[5]. The availability of fast amino acid sequence procedures for actin[5] led us to study the actin from *Physarum polycephalum* to assess how cytoplasmic actins differ between lower and higher eukaryotes. We report here results which show that *Physarum* plasmodia, in contrast to non-muscle cells of higher vertebrates[5–7], contain only one cytoplasmic actin species. This actin is more closely related to mammalian cytoplasmic actins than these actins are to skeletal muscle actin. The high degree of structural conservation of the actin sequence and structure during eukaryotic evolution is illustrated by the absence of any amino acid deletions or additions to the polypeptide chain beyond residue 2. Furthermore, the distribution of the proline residues remains unchanged and substitutions of amino acid residues with charged side chains are very rare, indicating a high conservation of the surface topography of the actin molecule.

Actin was purified from an acetone powder prepared from *Physarum* plasmodia. A low salt extract was chromatographed on deoxyribonuclease I immobilised on Sepharose 4B as described elsewhere[5,8]. The resulting actin preparation was at least 95% pure as judged by polyacrylamide gel electrophoresis in the presence of dodecyl sulphate, where *Physarum* actin coelectrophoreses with mammalian actins. In addition, isoelectric focusing analysis in the presence of urea and NP40 revealed a single protein species, focusing, in agreement with previous studies[9], very close to although at a slightly more acidic *p*H than rabbit skeletal muscle actin. *Physarum* actin was processed through performic acid oxidation and tryptic digestion as described for other actins[5,10]. Peptides were separated by the preparative two-dimensional 'fingerprint' system on Whatman 3MM paper which we have described in detail previously[5,10,11]. The insoluble tryptic 'core' was digested with thermolysin and the resulting peptides purified as described elsewhere[5]. This system accounts by tryptic and thermolytic peptides for the full amino acid sequence of the actin polypeptide chain[5]. All peptides were characterised by their electrophoretic and chromatographic mobilities, their amino acid composition and their behaviour in the Offord plot[12]. Peptides of > 10 amino acid residues were further fragmented by secondary enzymatic digestion and the isolated fragments characterised as above. Amino acid substitutions indicated in the resulting proteolytic fragments were localised by manual dansyl-Edman degradation and/or by degradation with leucine aminopeptidase and carboxypeptidase Y. A full description of the actin sequence methodology is presented elsewhere[5].

Figure 1 shows the complete amino acid sequence of *Physarum* actin. The order of the tryptic and thermolytic peptides in the actin polypeptide chain is based on homology with the rabbit skeletal muscle actin sequence[13]. The polypeptide chain contains 375 amino acid residues although the numbering system indicates only 374. This is because a serine residue following residue 234 has been found in all cytoplasmic actins so far studied[5,14] and has probably been overlooked in the original rabbit skeletal muscle actin sequence[13,14]. To avoid renumbering of the previously documented amino acid exchange past this residue, we agree with the proposal[14] to indicate this residue as serine 234a.

Table 1 summarises those amino acid exchanges which differentiate *Physarum* actin (Fig. 1) from mammalian cyto-

Fig. 1 Amino acid sequence of actin from plasmodium of *Physarum polycephalum*. X indicates a blocking group which is probably the acetyl group found in other actins[15]. His$^{\tau\mathrm{Me}}$ is N^{τ}-methylhistidine. For methods on sequence determination, see ref. 5 and text. Residues which are underlined differ from the corresponding amino acid residue in mammalian γ-cytoplasmic actin[5]. Alignment of the *Physarum* actin peptides was based upon comparison with the rabbit skeletal muscle actin sequence[13].

```
                                      10                                      20
X-Glu-Gly-Glu-Asp-Val-Gln-Ala-Leu-Val-Ile-Asp-Asn-Gly-Ser-Gly-Met-Cys-Lys-Ala-Gly-
                                      30                                      40
  Phe-Ala-Gly-Asp-Asp-Ala-Pro-Arg-Ala-Val-Phe-Pro-Ser-Ile-Val-Gly-Arg-Pro-Arg-His-
                                      50                                      60
  Thr-Gly-Val-Met-Val-Gly-Met-Gly-Gln-Lys-Asp-Ser-Tyr-Val-Gly-Asp-Glu-Ala-Gln-Ser-
                                      70          τMe                         80
  Lys-Arg-Gly-Ile-Leu-Thr-Leu-Lys-Tyr-Pro-Ile-Glu-His-Gly-Ile-Val-Thr-Asn-Trp-Asp-
                                      90                                     100
  Asp-Met-Glu-Lys-Ile-Trp-His-His-Thr-Phe-Tyr-Asn-Glu-Leu-Arg-Val-Ala-Pro-Glu-Glu-
                                     110                                     120
  His-Pro-Val-Leu-Leu-Thr-Glu-Ala-Pro-Leu-Asn-Pro-Lys-Ala-Asn-Arg-Glu-Lys-Met-Thr-
                                     130                                     140
  Gln-Ile-Met-Phe-Glu-Thr-Phe-Asn-Thr-Pro-Ala-Met-Tyr-Val-Ala-Ile-Gln-Ala-Val-Leu-
                                     150                                     160
  Ser-Leu-Tyr-Ala-Ser-Gly-Arg-Thr-Thr-Gly-Ile-Val-Met-Asp-Ser-Gly-Asp-Gly-Val-Ser-
                                     170                                     180
  His-Thr-Val-Pro-Ile-Tyr-Glu-Gly-Tyr-Ala-Leu-Pro-His-Ala-Ile-Leu-Arg-Leu-Asp-Leu-
                                     190                                     200
  Ala-Gly-Arg-Asp-Leu-Thr-Asp-Tyr-Leu-Met-Lys-Ile-Leu-Thr-Glu-Arg-Gly-Tyr-Ser-Phe-
                                     210                                     220
  Thr-Thr-Thr-Ala-Glu-Arg-Glu-Ile-Val-Arg-Asp-Ile-Lys-Glu-Lys-Leu-Ala-Tyr-Val-Ala-
                                     230             234a                    239
  Leu-Asp-Phe-Glu-Gln-Glu-Met-Gln-Thr-Ala-Ala-Ser-Ser-Ser-Ala-Leu-Glu-Lys-Ser-Tyr-
  240                                     250
  Glu-Leu-Pro-Asp-Gly-Gln-Val-Ile-Thr-Ile-Gly-Asn-Glu-Arg-Phe-Arg-Cys-Pro-Glu-Ala-
  260                                     270
  Leu-Phe-Gln-Pro-Ser-Phe-Leu-Gly-Met-Glu-Ser-Ala-Gly-Ile-His-Glu-Thr-Thr-Tyr-Asn-
  280                                     290
  Ser-Ile-Met-Lys-Cys-Asp-Val-Asp-Ile-Arg-Lys-Asp-Leu-Tyr-Gly-Asn-Val-Val-Leu-Ser-
  300                                     310
  Gly-Gly-Thr-Thr-Met-Phe-Pro-Gly-Ile-Ala-Asp-Arg-Met-Gln-Lys-Glu-Leu-Thr-Ala-Leu-
  320                                     330
  Ala-Pro-Ser-Thr-Met-Lys-Ile-Lys-Ile-Ile-Ala-Pro-Pro-Glu-Arg-Lys-Tyr-Ser-Val-Trp-
  340                                     350
  Ile-Gly-Gly-Ser-Ile-Leu-Ala-Ser-Leu-Ser-Thr-Phe-Gln-Gln-Met-Trp-Ile-Ser-Lys-Glu-
  360                                     370
  Glu-Tyr-Asp-Glu-Ser-Gly-Pro-Ser-Ile-Val-His-Arg-Lys-Cys-Phe
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Table 1 Positions in the amino acid sequence at which exchanges have been detected between actins from *Physarum polycephalum*, mammalian non-muscle tissue and rabbit skeletal muscle

Position	Mammalian cytoplasmic actin β	γ	*Physarum polycephalum* actin*	Rabbit skeletal muscle actin†
1	Absent		Glu	Asp
2	Asp	Glu	Gly	Glu
3	Asp	Glu	Glu	Asp
4	Asp	Glu	Asp	Glu
5	Ile		Val	Thr
6	Ala		Gln	Thr
10	Val	Ile	Ile	Cys
16	Met		Met	Leu
17	Cys		Cys	Val
41	Gln		Thr	Gln
76	Val		Val	Ile
103	Val		Val	Thr
129	Thr		Thr	Val
153	Met		Met	Leu
160	Thr		Ser	Thr
162	Thr		Thr	Asn
176	Leu		Leu	Met
201	Thr		Thr	Val
217	Cys		Ala	Cys
225	Gln		Gln	Asn
228	Ala		Gln	Ala
234a	Ser		Ala	Ser
259	Ala		Ala	Thr
266	Leu		Leu	Ile
271	Cys		Ala	Ala
278	Phe		Tyr	Tyr
286	Val		Val	Ile
294	Ala		Gly	Ala
296	Thr		Val	Asn
298	Leu		Leu	Met
305	Tyr		Phe	Tyr
316	Ile		Leu	Ile
357	Ser		Ser	Thr
359	Gln		Glu	Gln
364	Ser		Ser	Ala

Data on the amino acid sequences of the mammalian cytoplasmic actins are taken from previous studies[5,10], those for rabbit skeletal muscle actin are derived from ref. 13 with minor corrections[5,14]. Amino acid residues given in separate columns for the mammalian cytoplasmic actins are found in the β and γ forms, respectively[5,10]. The numbering system using 234a is taken from ref. 14 (see also ref. 5 and text).

* Residues underlined indicate the 17 positions where *Physarum* actin differs from mammalian γ cytoplasmic actin.

† Residues underlined indicate the 25 positions where mammalian skeletal muscle actin differs from mammalian cytoplasmic actin.

plasmic actins[5] and from rabbit skeletal muscle actin[13], with the latter sequence containing the few minor corrections recently proposed[5,14]. Like all other actins, *Physarum* actin contains 3-methylhistidine at position 73. We have not been able to detect *N*-methyllysine residues in *Physarum* actin, although they have been reported to occur in *Acanthamoeba* actin[15].

The results presented in Table 1 indicate the strict conservation of the actin structure during evolution. *Physarum* actin and mammalian γ-cytoplasmic actin differ in only 4% of their primary structure (see the 17 positions indicated in Table 1). Comparison with rabbit skeletal muscle actin reveals 8% differences. We previously reported a 7% difference in primary structure in the comparison of mammalian cytoplasmic actins and skeletal muscle actin[5] (see the 25 positions indicated in Table 1). Beyond the six N-terminal residues which are highly variable in all actins so far studied[10,16], *Physarum* actin differs from mammalian cytoplasmic actins in only 12 residues out of a total of 369 residues (a difference of approximately only 3.2%). As was the case for different mammalian actins[5,10,16], the few amino acid exchanges involving charged residues are concentrated in the far N-terminal region of the actin polypeptide chain. In addition, however, *Physarum* actin shows a single Gln→Glu substitution (residue 359) past the first four N-terminal residues. The partial sequence data[4] on *Acanthamoeba* actin indicate another rare substitution involving a charged residue, that is, residue 228 shows an Ala→His exchange. As *Physarum* actin is characterised by a Gln in this position, it seems possible that this residue is rather variable. In the remainder of the currently available sequence data *Acanthamoeba* actin (ref. 4 and Elzinga cited in ref. 2) and *Physarum* actin are rather similar and so far only five amino acid substitutions (residues 76, 103, 106, 228 and 359) have been found.

Physarum actin differs by isoelectric focusing analysis from mammalian cytoplasmic β and γ actins and behaves very similarly to skeletal muscle actin (α-actin)[9]. The sequence data support this result in general, as Glu 359 gives for *Physarum* actin and skeletal muscle actin the same total number of acidic and basic amino acid residues, whereas the more basic mammalian cytoplasmic actins show one less acidic residue (for a discussion of isoelectric points of different actins see refs 5–7, 10). However, we stress that skeletal muscle actin has one more aspartic acid residue and one less glutamic acid residue than *Physarum* actin. Therefore, one could expect a slightly higher isoelectric point for *Physarum* actin, whereas a slightly lower isoelectric point is found experimentally. We cannot explain this small difference, although it could be due to some subtle influence by amino acid changes next to some charged residues, or to the interruption of the four continuous acidic residues at the N-terminus of muscle actin on transition to *Physarum* actin.

The amino acid sequences of several actins from higher vertebrates[5,10] together with the sequence of *Physarum* actin explain why several antiactin antibodies show no species specificity and cover species as divergent as man and *Dictostelium*[17] or *Physarum* (K.W., unpublished observation).

Are the 17 observed amino acid exchanges between *Physarum* actin and mammalian cytoplasmic actin functionally important or are they solely the result of evolutionary drift? Studies by Korn *et al.*[2,18] show that the vertebrate cytoplasmic F-actins differed from the *Acanthamoeba* F-actin in their ability to activate the Mg^{2+} ATPase of rabbit skeletal muscle heavy meromyosin. Thus, our working hypothesis is that some of the amino acid exchanges found between *Physarum* and mammalian cytoplasmic actins will have an important role in the interaction between actin and myosin. Future experiments are aimed at the identification of these residues.

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Bromodeoxyuridine mutagenesis in mammalian cells is stimulated by thymidine and suppressed by deoxycytidine

THE most widely accepted explanation for the mutagenicity of 5-bromodeoxyuridine (BUdR) is that mutations occur as the result of occasional mispairing of the analogue during DNA replication[1,2], although the correlation between mutagenesis and the amount of bromouracil (BU) incorporated into DNA has been questioned[3,4]. We have previously shown that BUdR mutagenesis in Syrian hamster cells (measured by the induction of ouabain-resistant (OB^r) or 6-thioguanine-resistant (TG^r) mutants) is determined by the concentration of BUdR in the medium rather than the amount of BU in DNA[5]. This suggested that BUdR nucleotides are involved in BUdR mutagenicity in mammalian cells[5] (see also ref. 4). BUdR triphosphate (BUdRTP) inhibits the ribonucleotide reductase-catalysed reduction of cytidine diphosphate to deoxycytidine (CdR) diphosphate[6], as does thymidine (TdR) triphosphate (dTTP)[7,8]. Therefore, we have investigated the effects of CdR and TdR on BUdR mutagenesis, and report here that the former suppresses and the latter stimulates this process.

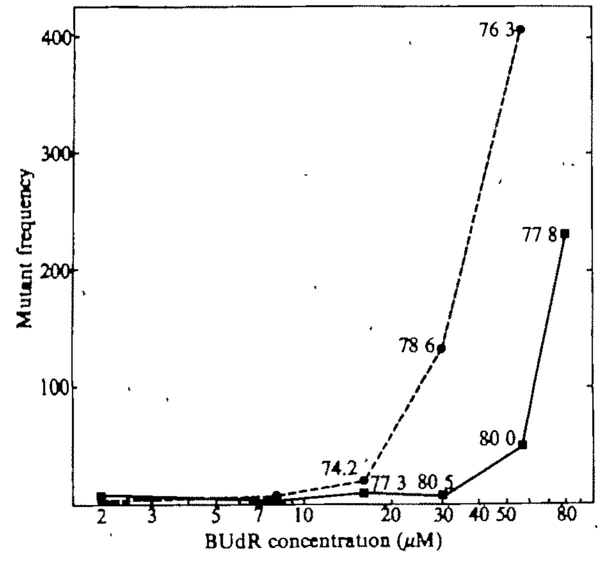
The cell lines used have been previously described. RPMI 3460 is a pigmented Syrian hamster melanoma line[9], and HAB-2E is a line derived from 3460 by selection for viability with all the thymine residues in nuclear DNA replaced by BU[5,10]. Most of the experiments were carried out with HAB-2E cells and OB^r as the marker for mutagenesis. Table 1 (expt 1) shows that the addition of 1 μM CdR decreased BUdR mutagenicity and that the maximum decrease was obtained with 10 μM CdR. The addition of 10 μM CdR also decreased BU incorporation into DNA (BU substitution), presumably due to the intracellular conversion of the CdR to TdR nucleotides[12]. However, the maximum suppression of mutagenicity was observed when the CdR concentration was increased from 3 to 10 μM, over which range there was no change in BU substitution. Similar results were obtained (Table 1, expt 2) in the presence of aminopterin, which completely blocks the conversion of CdR to TdR nucleotides[12]. Thus, CdR suppresses BUdR mutagenicity without being converted to TdR nucleotides and without altering the amount of BU in DNA.

Table 1 Suppression of BUdR mutagenesis by CdR

Aminopterin	Additive (μM) BUdR	TdR	CdR	BU substitution (%)	Mutant frequency
Expt 1					
–	–	30	–	–	<0.5
–	30	–	–	72.3	349
–	50	12.5	–	67.7	1,194
–	50	12.5	1	63.2	397
–	50	12.5	3	58.0	221
–	50	12.5	10	58.1	37
–	50	12.5	30	57.7	30
–	50	12.5	100	55.4	46
Expt 2					
–	–	30	–	–	<0.3
–	30	–	–	71.3	295
+	50	12.5	–	77.5	463
+	50	12.5	1	ND	252
+	50	12.5	3	ND	262
+	50	12.5	10	78.6	82
+	50	12.5	30	ND	83
+	50	12.5	100	ND	105

HAB-2E cells were maintained routinely in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (E medium). For mutagenicity assays, Falcon plastic tissue culture flasks (75 cm^2) were inoculated with 5×10^5 cells in 10 ml of E medium containing the indicated additives When aminopterin was used, it was present at 0.4 μM and 0.1 mM hypoxanthine was also added. At 3 and 6 d after inoculation, the medium was renewed with 50 ml fresh medium containing the same additives. After 1 week in BUdR-containing medium, the cells were collected and flasks (75 cm^2) were reinoculated with 5×10^5 cells in 10 ml of E medium containing 0.1 mM hypoxanthine and 16 μM TdR (HT medium). They were cultured in HT medium for 1 week to allow the expression of induced mutations, and then collected and tested for drug resistance. Cells were protected at all times from exposure to light with wavelengths below 550 nm (ref. 11), because of the increased photosensitivity of cells with BU-substituted DNA. During the exposure of cells to BUdR for mutagenesis, the level of BU incorporated into nuclear DNA was determined as previously described[5]. The amount of BU in DNA is expressed as 'BU substitution', defined as the % of thymine residues in nuclear DNA replaced by BU. The results are the mean of four determinations per sample; the s.e.m was generally $< \pm0.5$. The frequency of OB^r mutants was determined by inoculating 3–5 tissue culture dishes (150 mm diameter) each with 10^6 cells in E medium containing 0.9 mM OB. Plating efficiencies were determined by inoculating 3 dishes (60 mm diameter) each with 250 cells in E medium alone. Seven or eight days after inoculation, the dishes were fixed with methanol and stained with Giemsa for colony counting. The mutant frequencies are expressed as the number of OB^r colonies per 10^6 cells and are corrected for the plating efficiency of the cells in the absence of OB. ND, not determined.

Fig. 1 Effect of BUdR concentration on the suppression of mutagenicity by CdR. HAB-2E cells were grown in E medium containing 0.1 mM hypoxanthine and 0.4 μM aminopterin, plus BUdR and TdR in a 4 : 1 ratio. The cells were grown either in the presence (■) or absence (●) of 200 μM CdR. The frequency of OB^r mutants was determined and expressed as described in Table 1. The numbers next to the data points indicate the levels of BU substitution for those points, as described in Table 1.



The effect of increasing the BUdR concentration in the presence of a fixed concentration of CdR was also determined (Fig. 1). HAB-2E cells were exposed to BUdR and TdR in a 4 : 1 ratio in the presence of aminopterin. In the absence of CdR, BUdR mutagenicity occurred when the BUdR concentration exceeded 10 μM. However, in the presence of CdR, BUdR mutagenicity did not occur until the BUdR concentration exceeded 30 μM. The increase in mutant frequency with increasing BUdR seemed to occur at approximately the same rate in the presence of CdR as in its absence, although at higher BUdR concentrations. Apparently, a 2.5-fold increase in BUdR concentration is sufficient to overcome the suppression of mutagenicity by CdR completely. Note that none of these effects was associated with changes in BU substitution.

As dTTP, like BUdRTP, can perturb CdR metabolism, the effects of TdR on BUdR mutagenicity were tested. Table 2 (expt 1) shows that the addition of 10 μM TdR to 10 μM BUdR (a

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relatively nonmutagenic concentration) increased the frequency of mutants while decreasing BU substitution. The addition of 100 μM TdR resulted in a similar stimulation of mutagenesis, even though the BU substitution dropped to a very low level. In addition, the stimulation by TdR was suppressed by CdR. Table 2 (expt 2) shows that TdR was also stimulatory to mutagenesis in the presence of aminopterin. CdR again suppressed not only BUdR mutagenicity but also TdR stimulation, and the CdR effects were not associated with changes in BU substitution. Because TdR stimulated mutagenesis, the mutagenicity of TdR alone was tested, but, up to 700 μM, was nonmutagenic (Table 2, expt 1). (The demonstration that TdR stimulated BUdR mutagenesis in these cells is at variance with studies on prokaryotes, in which thymine suppresses BU mutagenesis.)

Table 2 Stimulation of BUdR mutagenesis by TdR

Aminopterin	Additive (μM) BUdR	TdR	CdR	BU substitution (%)	Mutant frequency
Expt 1					
–	–	30	–	–	<0.4
–	30	–	–	68.1	119
–	30	–	200	36.9	22
–	10	–	–	31.0	4.5
–	10	–	200	18.3	<0.3
–	10	10	–	26.2	19
–	10	10	200	15.3	0.7
–	10	30	–	16.7	13
–	10	30	200	11.9	1.7
–	10	100	–	7.4	22
–	10	100	200	5 6	0.7
–	–	700	–	–	<0.3
Expt 2					
–	30	–	–	65.3	281
+	10	–	–	96.7	31
+	10	–	200	96.7	6.0
+	10	10	–	40.8	39
+	10	10	200	38.7	0.4
+	10	30	–	19.5	94
+	10	30	200	20.5	3.9
+	10	100	–	8.4	93
+	10	100	200	7.2	1.4

HAB-2E cells were assayed for mutagenicity as described in Table 1, as are methods for determination of BU substitution and mutant frequency.

Experiments were carried out, using resistance to 18 μM TG as the marker, to determine whether the above results were applicable to mutations other than OB^r. Experiments were also carried out using the parental melanoma line 3460, to determine whether the above conclusions were applicable to wild-type cells as well as HAB-2E. The results of these experiments agreed completely with those described above (data not shown).

The results presented above suggest that interactions involving small molecules play a central part in BUdR mutagenesis in mammalian cells. We have shown that (1) CdR suppresses BUdR mutagenicity, (2) CdR suppression can occur without the conversion of CdR to TdR nucleotides and without any change in the amount of BU in DNA, (3) TdR stimulates the mutagenicity of BUdR, while decreasing the amount of BU in DNA, and (4) the stimulation by TdR is suppressed by CdR. These results were observed for two markers of mutagenesis and for two cell lines.

The molecular mechanisms underlying the effects of CdR and TdR on BUdR mutagenesis remain to be elucidated. However, both BUdRTP and dTTP inhibit the ribonucleotide reductase-catalysed reaction which generates CdR nucleotides. Thus, BUdR mutagenicity in mammalian cells, and its enhancement by TdR, may involve the perturbation of CdR metabolism. As mutagenesis can occur even in the presence of very high concentrations of CdR, the results cannot be explained by simple CdR starvation. In addition, as TdR by itself is not mutagenic, it seems that simply perturbing CdR metabolism in the absence of BUdR does not cause mutations. (Our results do not agree with a recent report of mutagenicity by TdR alone[13].) Apparently, some BUdR is necessary to TdR stimulation of mutagenesis to occur, but only a relatively small amount of BUdR is sufficient.

One possible mechanism for the role of CdR metabolism in BUdR mutagenesis is that mutagenesis could involve an 'error-prone' repair system[14] induced by the perturbation of CdR metabolism. It is also possible that altered CdR metabolism could cause an increased error frequency during DNA replication, due to allosteric changes in DNA polymerase itself (compare ref. 4) or to an imbalance of the deoxyribonucleoside triphosphates[13,15]. Although our results demonstrate that BUdR mutagenicity involves effects at sites outside DNA, they do not indicate whether there must also be some BU present in DNA to act as a substrate for any mutagenic functions induced.

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Stimulation of DNA synthesis by tumour promoter and pure mitogenic factors

12-*O*-TETRADECANOYL PHORBOL-13-ACETATE (TPA), a potent tumour-promoting agent, stimulates the proliferation of various cell types, including fibroblasts, in cell culture (see ref. 1 for review). Recent studies have demonstrated that there is a close correlation between the potency of phorbol esters for promoting skin tumours and their ability to stimulate DNA synthesis in cultured cells[2]. The growth-stimulating activity of TPA is completely dependent on the presence of serum in the nutrient medium. This requirement for serum has been demonstrated in cultures of mouse epidermal cells[3] and fibroblastic cells[4,5]. The role of serum in the cellular response to TPA remains unresolved. We report here that serum can be replaced by polypeptide hormones like epidermal growth factor (EGF)[6], and insulin and by the growth factor isolated from the conditioned medium of SV40 BHK cells (fibroblast-derived growth factor, FDGF)[7]. Low concentrations of TPA and the growth-

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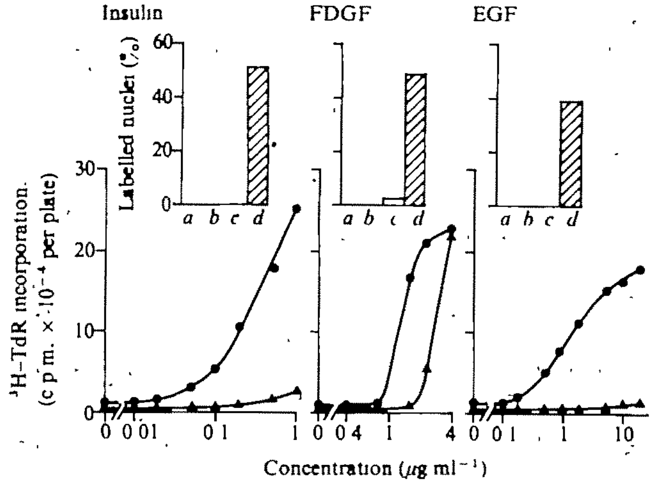
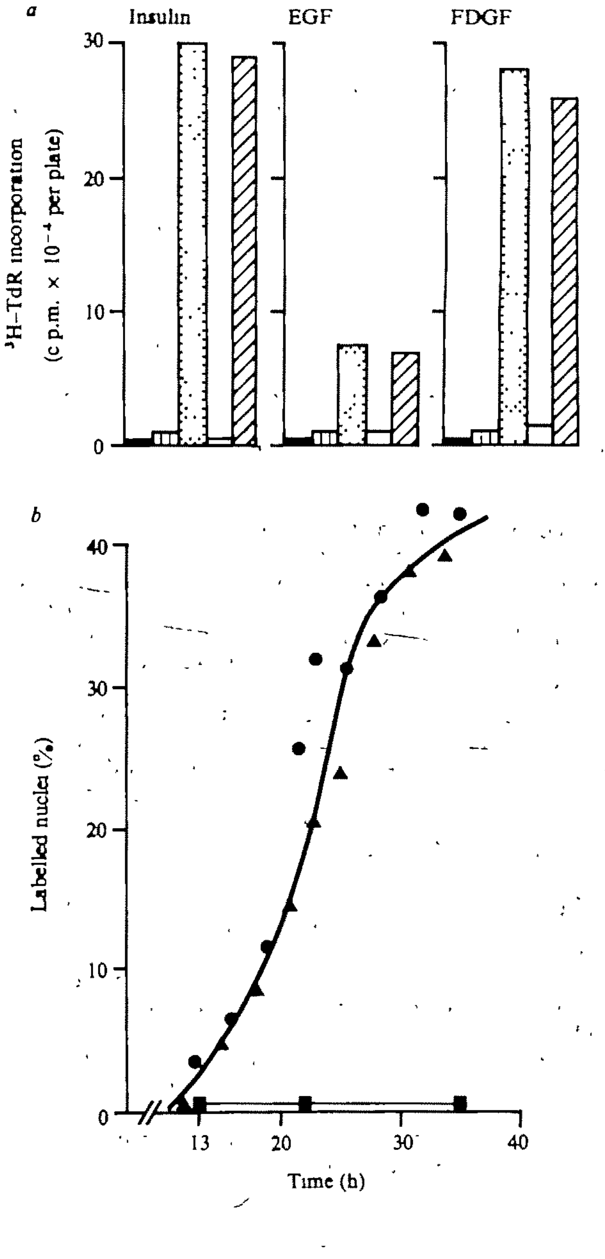


Fig. 1 Effect of insulin, FDGF or EGF on DNA synthesis in the absence (▲) or in the presence of 0.1 μg ml^{-1} TPA (●). Cultures were exposed to the peptide with or without TPA in 2 ml of a 1:1 mixture of Dulbecco's modified Eagles' medium (DMEM) and Waymouth's[14] medium containing ^{3}H-TdR (0.5 μCi ml^{-1}; 0.5 μM). After 40 h at 37 °C, the radioactivity incorporated into acid-precipitable material was measured as previously described[15] (10% foetal calf serum produced ^{3}H-TdR incorporation of 26.5 × 10^4, 27.5 × 10^4 and 30.7 × 10^4 c.p.m. respectively, for the experiments involving insulin, FDGF and EGF). All points are the average of duplicate plates. Stock 3T3 cells were passaged at 3-d intervals in DMEM containing 10% foetal calf serum–100 U ml^{-1} penicillin and 100 ng ml^{-1} streptomycin in a humidified 10% CO_2 atmosphere at 37 °C. In all experiments, cell suspensions were plated in 30-mm Nunc dishes in DMEM supplemented with 10% foetal calf serum, re-fed with the same medium after 3 d, and kept for at least a further 5 d. The cultures of 3T3 cells used were arrested in the G_1/G_0 phase of the cell cycle. The cultures were washed twice with DMEM at 37 °C to remove residual serum immediately preceding the experiment. Bovine insulin 26 IU mg^{-1} was obtained from Sigma; EGF was supplied by Dr S. Cohen, and FDGF was isolated and purified from the medium conditioned by SV40 BHK cells as described previously[7]. TPA was obtained from Consolidated Midland Corporation (Brewster, New York). Stock solutions of 10 mg ml^{-1} TPA were made up in acetone and kept at −20 °C. Equivalent concentrations of acetone were used in all controls. Insets: The experimental conditions were as described above except that the DMEM/Waymouths medium contained ^{3}H-TdR (5 μCi ml^{-1}; 0.2 μM) and the cultures were fixed for autoradiography as described previously[15]. The bars represent the percentage of nuclei incorporating the ^{3}H-TdR: *a*, no addition; *b* 100 ng ml^{-1} TPA, *c*, peptide; *d*, 100 ng ml^{-1} TPA + peptide. The concentrations of insulin, FDGF and EGF were 1 μg ml^{-1}, 1.8 μg ml^{-1} and 10 ng ml^{-1} respectively. Foetal bovine serum produced 79%, 79% and 89% autoradiographically labelled nuclei respectively, in the experiments involving insulin, FDGF and EGF.

factors stimulate DNA synthesis in quiescent cultures of mouse and human cells in completely serum-free medium.

Figure 1 shows that TPA interacts synergistically with insulin, EGF and FDGF in stimulating ^{3}H-TdR incorporation into acid-precipitable material by quiescent cultures of 3T3 cells. The effects are obtained in entirely synthetic, serum-free medium. The interaction between the tumour promoter and EGF or insulin is particularly striking, because neither of these substances produces a substantial stimulation of DNA synthesis by itself. EGF and TPA reproducibly gave a considerable increase in DNA synthesis in eight independent experiments although the magnitude of the increase varied. The inserts in Fig. 1 show that similar results are obtained when the percentage of labelled nuclei incorporating ^{3}H-TdR was measured, instead of the total ^{3}H-TdR incorporation. In the presence of a fixed concentration of insulin, TPA stimulates DNA synthesis in a dose-dependent manner (results not shown). The lowest

Fig. 2 Effect of sequential incubation of TPA and growth-stimulating factors on DNA synthesis in quiescent 3T3 cells. *a*, Quiescent cultures of 3T3 cells were produced as in Fig 1. Continuous incubations were washed eight times in DMEM at 37 °C before the experiment. Black bar, cultures were incubated continuously in 200 ng ml^{-1} TPA for 40 h; vertical striped bar, cultures were incubated continuously in peptide for 40 h; dotted bar, cultures were incubated for 40 h with 200 ng ml^{-1} TPA and peptide; plain bar, cultures were washed twice with DMEM at 37 °C, incubated 20 min with hormone, washed six times in DMEM at 37 °C and incubated for 40 h with 200 ng ml^{-1} TPA; oblique striped bar, cultures were washed twice in DMEM at 37 °C, incubated 20 min with 200 ng ml^{-1} TPA, washed six times in DMEM then incubated for 40 h with hormone. The final incubation for all cells was in 2 ml 1:1 Waymouths–DMEM containing ^{3}H-TdR (0.5 μCi ml^{-1} 0.5 μM). Incorporation of ^{3}H-TdR into acid-precipitable material was measured as in Fig. 1. Concentrations of peptides were insulin 1 μg ml^{-1}, FDGF 0.7 μg ml^{-1}, EGF 10 ng ml^{-1}. *b*, Percentage of nuclei labelled autoradiographically after continuous incubation with TPA and insulin (●), TPA or insulin alone (■), or by two washes with DMEM at 37 °C followed by 20 min incubation with TPA, six washes of DMEM at 37 °C, and then incubation with 1 μg ml^{-1} insulin (▲). All continuous incubations were washed eight times at the start of the experiment. The final incubation of all cultures was in 2 ml of 1:1 Waymouths–DMEM containing ^{3}H-TdR (5 μCi ml^{-1} 0.2 μM). All points are in duplicate. Autoradiography was performed as described previously[15]. 10% serum produced 86% labelled nuclei at 35 h



concentration of TPA needed to show synergism consistently with 1 μg ml^{-1} insulin was 5 ng mg^{-1}. In contrast to the effect of TPA, phorbol itself, tested either in the absence or presence of insulin, was completely inactive in stimulating DNA synthesis. The close analogue, 4-*O*-methyl-12-*O*-tetradecanoyl-phorbol-13-acetate, was slightly mitogenic (18% of the maximal effect of TPA with insulin) at high concentrations (1 μg ml^{-1}) and in the presence of 1 μg ml^{-1} insulin.

An interesting feature of the synergistic interaction between TPA with insulin is that a brief exposure (20 min) to the promoter was sufficient to render the cultures sensitive to the mitogenic effect of insulin, EGF or FDGF (Fig. 2*a*). Although the effect of TPA persisted even after thorough washing of the cell monolayer (six times with DMEM at 37 °C), it is not known whether the promoter binds to the cultures in a non-washable manner of whether it causes a lasting change in the culture[8]. Brief exposure of cultures to insulin, EGF or FDGF did not result in their becoming sensitive to the effects of TPA (Fig. 2*a*).

Table 1 Synergistic interaction between TPA and insulin on DNA synthesis by quiescent cultures of human embryo lung fibroblasts and 3T6 cells

	Cell type	
	Human embryo lung fibroblasts	3T6
No addition	4.2%	1.7%
10 ng ml^{-1} TPA	7.0%	27.0%
100 ng ml^{-1} insulin	3 6%	8 3%
10 ng ml^{-1} TPA + 100 ng ml^{-1} insulin	22.0%	72.0%

Swiss 3T6 cells were maintained in DMEM containing 10% fetal calf serum and subcultured at 3-d intervals. Quiescent cultures in 30-mm dishes were obtained as previously described[16] Experimental conditions for autoradiography were as in Fig. 1. Primary cultures of human lung embryo lung fibroblasts (provided by Dr F. Balkwill), were maintained in 10% fetal calf serum 90% RPMI 1640 and subcultured once every 7 d. For the above experiment, with 7th passage cells, cells were subcultured into 30-mm plates containing RPMI 1640 supplemented with 0.5% foetal calf serum depleted by 7-d incubation with parallel cultures of human embryo fibroblasts, and left for 5 d They were then washed twice and left in serum-free RPMI 1640 for 4 d. Cells were incubated at 37 °C in RPMI 1640 containing ^{3}H-TdR (5 μCi ml^{-1} 0.2 μM) and different additions for 40 h. Autoradiography was carried out as in Fig 1.

In 3T3 cells, TPA and insulin stimulated-DNA synthesis after a lag of approximately 13 h (Fig. 2*b*). Addition of whole serum stimulated DNA synthesis after a similar lag. The time course of DNA synthesis produced by brief exposure to TPA followed by washing and insulin addition is similar to that produced when insulin and TPA are incubated continuously with the cells (Fig. 2*b*).

The striking synergistic interaction between growth-promoting polypeptides and TPA was also observed when cell multiplication was monitored over a period of several days (Fig. 3). In these experiments, a certain amount of depleted serum (see legend to Fig. 3) was required to prevent cell detachment. Neither TPA nor insulin added to such cultures caused a substantial increase in growth, however, both insulin and TPA together caused a 2–3-fold increase in cell number. Similarly, although EGF and insulin together caused a two-fold increase in cell number, the addition of the tumour promoter with both insulin and EGF resulted in four-fold increase in cell number.

The synergistic interaction between insulin and tumour promoters was also observed in quiescent cultures of early passage human embryo fibroblasts as well as murine 3T6 cells (Table 1). Insulin potentiated the effect of low concentrations of TPA in both cell types. TPA tested at higher concentrations (100–300 ng ml^{-1}) produced a substantial increase in the labelling index of both cell types (results not shown).

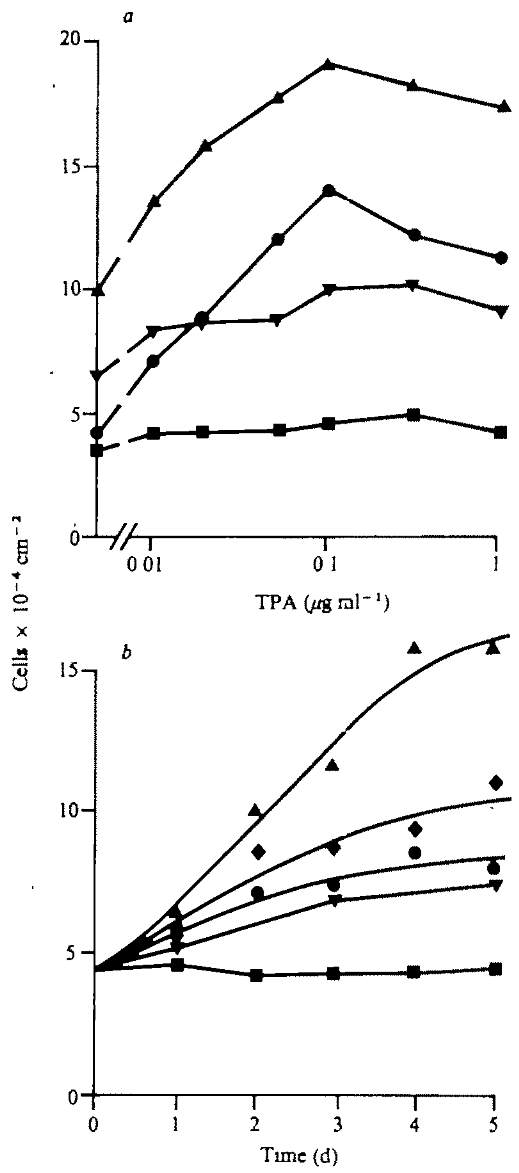


Fig. 3 Effect of insulin, EGF and TPA on cell density when added to quiescent cultures of Swiss 3T3 cells. Culture conditions were as in Fig. 1. Medium from parallel cultures was removed and one-third its volume of Waymouth's medium added to replace low molecular weight nutrients that might have become depleted TPA, insulin and EGF were then added to give final concentrations as in the figures Cell number determinations were made on duplicate plates For each culture, two aliquots of cell suspension, produced by thorough trypsinisation were counted on a Coulter counter. *a*, Effect of different concentrations of TPA on cell density after 5 d of exposure in the absence (■) or in the presence of (▼) 10 ng ml^{-1} EGF; (●) 1 μg ml^{-1} insulin, (▲) 10 ng ml^{-1} EGF and 1 μg ml^{-1} insulin Initial cell density was 4.0×10^4 cells cm^{-2} *b*, Time course of the effect on cell density produced by addition of: (■) 200 ng ml^{-1} TPA, 1 μg ml^{-1} insulin, or 10 ng ml^{-1} EGF alone, (▼) 10 ng ml^{-1} EGF and 200 ng ml^{-1} TPA; (●) 1 μg ml^{-1} insulin and 200 ng ml^{-1} TPA; (◆) 1 μg ml^{-1} insulin and 10 ng ml^{-1} EGF, (▲) 1 μg ml^{-1} insulin, 10 ng ml^{-1} EGF and 200 ng ml^{-1} TPA together. Initial cells density was 4.4×10^4 cells cm^{-2}.

The present experiments indicate that there is a striking synergistic interaction between TPA and growth factors in stimulating DNA synthesis in quiescent cultures of mouse and human fibroblasts maintained in serum-free medium Chemically defined conditions are not only of importance for the study of the mechanism of action of TPA, but may also provide more reproducible assay conditions to screen for tumour promoters or anti-tumour promoters than systems using serum[2,13]. Our findings raise interesting questions regarding other important cellular effects of phorbol ester. It is now reasonable to ask whether in enhancing the expression of the transformed phenotype[9,10], or in blocking terminal differentiation in a variety of cell types[11,12], tumour promoters interact with growth-promoting factors added to the medium, present in serum or produced by the cells. Indeed, the possibility that promotion *in vivo* can be induced by an interaction between the promoter and growth factors should receive serious consideration.

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Direct resorption of bone by human breast cancer cells *in vitro*

OSTEOLYTIC bone metastases occur frequency in patients with advanced malignancy. Breast cancer, the most common malignant disease of women, metastasises to bone more frequently than to any other organ[1], and over 80% of patients with advanced breast cancer develop bone metastases[2–4]. The cellular and molecular mechanisms of bone destruction in patients with cancer are not clearly understood. It has been postulated that there may be two mechanisms, one which is mediated by osteoclasts and one which occurs independently of osteoclasts[5,6]. We show here that cultured human breast cancer cells have the capacity to resorb directly *in vitro*, independently of osteoclast stimulation.

Supernatant media collected from the human breast cancer cell line MCF-7 (ref. 7) were tested for bone-resorbing activity by culture with live and killed fetal rodent long bones previously incorporated with ^{45}Ca or ^{3}H-proline. The assay system for live bones has been described in detail previously[8,9]. The killed bones were devitalised by overnight exposure to UV light to leave a calcified acellular bone matrix. There is no glucose uptake by these bones and they do not respond to parathormone (PTH) or prostaglandins. Killing the bones allows assessment of the effect of exogenous cells on bone resorption independently of the effects of endogenous bone cells[10]. The bones were labelled with ^{45}Ca or ^{3}H-proline[11] by injection of the mothers 1–3 d before death. Mineral release and matrix resorption were assessed in the same way as for live bones. Experiments on matrix resorption were carried out with mouse bones rather than fetal rat bones because it was easier to incorporate the label into the former, probably because of the smaller volume of distribution of the isotope in the pregnant mice.

Table 1 shows that in three separate experiments supernatants from MCF-7 cell cultures caused the release of previously incorporated ^{45}Ca from live and dead fetal rat long bones. Similar results were obtained when the bones were co-cultured with the MCF-7 cells. Table 2 shows that the bone matrix was also resorbed by the MCF-7 cells and their conditioned media. In these experiments we determined the proportion of the radioactivity released into the media from the bones which was in the form of ^{3}H-hydroxyproline, because this latter component reflects bone collagen breakdown[11]. Media from individual bone cultures were pooled before chromatography on a Dowex column so that standard errors could not be calculated. There was very close agreement between the data obtained from release of total radioactivity and the data obtained from release of ^{3}H-hydroxyproline (Table 2).

Table 1 Effects of supernatant media from cultured human breast cancer cells (MCF−7) on mineral release from live and dead fetal rat long bones compared with paired bones treated with control media

Bones	^{45}Ca Release (treated /control ratios) Expt *A*	Expt *B*	Expt *C*
Live	1.39±0.13*	1.75±0.06*	1.96±0.10*
Dead	1.59±0.06*	1.41±0.09*	1.77±0.03*

MCF−7 cells were grown in monolayer culture in Dulbecco's modified Eagle medium (Gibco) with 10% heat-inactivated fetal calf serum. Cell-free supernatants were collected from cultures which were near confluency. The assay for bone resorption is based on the release of previously incorporated ^{45}Ca from fetal rodent long bones in organ culture[8,9]. Bone-resorbing activity was quantitated as the ratio of ^{45}Ca released into the media by test bones compared with ^{45}Ca released into the media from paired bones cultured in control media. Each bone was cultured for 48 h with 0.5 ml of media from the MCF-7 cultures. Values are means ±s.e.m. for 4 pairs of bone cultures.

*Significantly greater than 1.0, $P < 0.05$.

Table 3 shows that the supernatants from four other established breast cancer cell lines caused mineral release from devitalised fetal rat bones. Other normal and neoplastic cells had no effect on bone mineral release. The effects of MCF-7 cells on bone could not be ascribed to changes in *p*H. Resorption occurred in experiments where there was no change in the measured *p*H of the media, and also when the *p*H of the supernatant media was readjusted to 7.4 before the bones were cultured in the cell-free media.

Fig. 1 Effects of pharmacological inhibitors of osteoclast activity on bone resorption due to human breast cancer cells (MCF-7) and prostaglandin-E_2 (10^{-7} M). The bone-resorbing effects of the MCF-7 supernatants were not altered by cortisol (10^{-6} M) or phosphate (6 mM). In contrast, PGE_2-mediated bone resorption was inhibited by these drugs. Bone resorption is measured as per cent increase in ^{45}Ca release from bones cultured with MCF-7 culture supernatants or PGE_2 compared with paired bones cultured in identical media except for the MCF-7 supernatant or PGE_2. *a*, MCF-7; *b*, MCF-7 + phosphate; *c*, MCF-7 + cortisol; *d*, PGE_2; *e*, PGE_2 + phosphate; *f*, PGE_2 + cortisol.

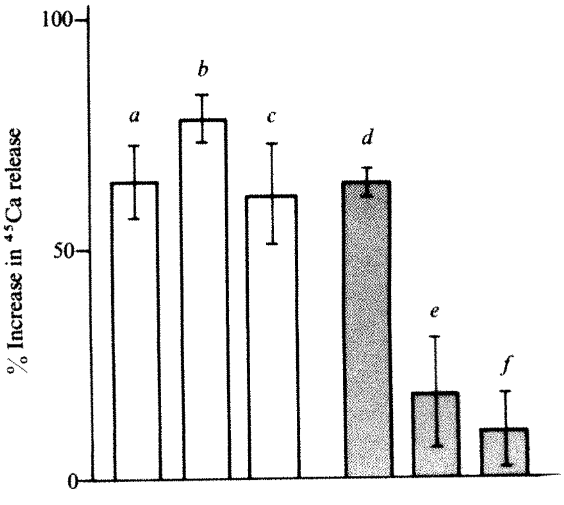

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Table 2 Effects of cultured human breast cancer cells (MCF-7) on matrix resorption of live and dead fetal rodent long bones labelled with ^{3}H-proline

Bones	Total radioactivity released (treated/control ratios)*	^{3}H-hydroxyproline released (treated/control ratios)
Live		
MCF-7 supernatants	1.67±0.10†	1.65
PTH (400 ng ml^{-1})	1.74±0.04†	1.75
Dead		
MCF-7 cells	2 26±0.26†	2.0
MCF-7 supernatants	1.38±0.09†	1.24

Fetal bones were labelled with ^{3}H-proline by injection of the mother 2 d and 1 d before death. Each bone was cultured with 0.6 ml media. At the end of the culture period, 100-μl aliquots of this media were removed and counted in a liquid scintillation counter so that treated-to-control ratios for total radioactivity released from pairs of bones could be obtained. The remaining media for each treatment group were pooled and applied to a Dowex column to separate ^{3}H-hyroxyproline from ^{3}H-proline[20]. All the radioactivity applied to this column was recovered in the fractions corresponding to where markers of proline and hydroxyproline were eluted. The duration of the bone cultures was 2 d. In some dishes, MCF-7 cells were co-cultured with the bones and in others supernatants taken from MCF-7 cultures near confluency were used

*Values are means ±s.e.m. for 4–12 pairs of bone cultures.

†Significantly greater than 1.0, $P < 0\ 05$.

Morphological features of resorbing live bones which were cultured with MCF-7 media were examined and compared with bones cultured with prostaglandin-E_2 (10^{-6}M). The bones which were cultured with prostaglandins showed many giant multinucleated osteoclasts adjacent to the endosteal bone surfaces. These bones were resorbed 80% more than corresponding paired control bones as measured by ^{45}Ca release. Similar features are found when bones are cultured with other humoral stimulators such as PTH, osteoclast-activating factor, active vitamin D metabolites[12], and thyroid hormones[13]. In contrast, bones which were cultured with supernatants from the MCF-7 cultures showed no detectable osteoclasts although there was a similar release of ^{45}Ca (83% increase in ^{45}Ca release compared with corresponding control).

To confirm that osteoclasts are not required for breast cancer cells to resorb bone, live bones were cultured with cortisol (10^{-6} M) and phosphate (6×10^{-3} M), both of which inhibit the effects of osteoclasts on bone[14,15]. Phosphate and cortisol had no effect on bones resorbing in response to MCF-7 media (Fig. 1). On the other hand, prostaglandin-stimulated bone resorption was inhibited in bone cultures to which cortisol or phosphate were also added. These data also indicate that the MCF-7 media resorbed bone independently of osteoclasts.

Prostaglandins could not have played any part in the effects that we have observed. They do not resorb devitalised bone[16], and phosphate and cortisol, which inhibited prostaglandin-stimulated bone resorption, had no effect on MCF-7 supernatants (Fig. 1). We confirmed that bone resorption stimulated by MCF-7 cells occurred independently of prostaglandins by culturing MCF-7 cells with indomethacin (10^{-5} M) on live bones. Indomethacin, which inhibits prostaglandin synthesis at this concentration, had no effect on the release of ^{45}Ca from these lives bones resorbed by the MCF-7 cells (data not shown). The conditioned media from MCF-7 cells do not contain sufficient immunoreactive prostaglandins of the E or $F_{2\alpha}$ types to cause bone resorption in this assay (data not shown).

Although our data show no evidence of osteoclast stimulation by breast cancer cells *in vitro*, this does not preclude a role for osteoclasts in metastasis formation and hypercalcaemia *in vivo*. It is possible that osteoclasts could be stimulated at metastatic sites by factors released by cells other than cancer cells. Local bone-resorbing factors such as prostaglandins[16] or osteoclast-activating factor[17] could be released by monocytes[18], activated lymphocytes[17] or fibroblasts as part of a cell-mediated immune response to the tumour. These effects would not be observed in our experiments.

Every breast cancer cell line that we tested resorbed bone *in vitro* (Table 3). This correlates with the clinical observation that nearly all patients with breast cancer will develop bone metastases if they live long enough with the disease[2–4]. Not all cultured neoplastic cells have this property (Table 3), but more work will be required to determine how frequently tumour cells in culture behave in this way.

Thus, these *in vitro* results suggest that there may be at least two mechanisms for the bone destruction which occurs in patients with malignant disease. Bone destruction may be osteoclast mediated and this is the probable mechanism in patients with myeloma[19] and with solid tumours who develop ectopic hormone syndromes. However, the studies described here show that some cancer cells can resorb bone directly independently of osteoclasts. In some patients it is possible that both mechanisms are relevant[5,6].

This work was supported by grants from the USPHS and the ACS. We thank Elissa Keogh, Mary Ann Kowalski and Monica Gondek for technical assistance, and Drs Lawrence Raisz and Peter Sherline for advice. MCF-7 cells and other human breast

Table 3 Effects of cell culture supernatants on mineral release from devitalised fetal rat long bones compared with paired bones cultured with control media

Cell line	Source	Clinical data	^{45}Ca release (treated/control ratios)
MCF−7	Malignant pleural effusion	Not known	1 42±0 07*
MDA-MB-157	Malignant pleural effusion	Not known	1 34±0 08*
MDA-MB-231	Malignant pleural effusion	Not known	1.54±0.13*
MDA-MB-330	Malignant pleural effusion	Bone metastases	1 43±0.09*
MDA-MB-134	Malignant pleural effusion	Bone metastases	1.35±0.13*
3T3 Cells	Mouse fibroblasts	No abnormality	1.08±0.05
Normal lymphocytes	Human peripheral blood	No abnormality	1.05±0.03
Chronic lymphocytic leukaemia lympocytes	Human peripheral blood	Osteopenia, normal serum calcium	0.96±0.03
Ovarian cancer	Human primary tumour	Hypercalcaemia, no bone metastases	0.99±0.05
Lung cancer	Human primary tumour	Hypercalcaemia, no bone metastases	0.90±0 12
Chondroblastoma	Human primary tumour	No skeletal abnormality	1.02±0.03

The duration of the bone cultures was 2 d. Supernatants were taken from tumour cells and 3T3 cells which were near confluency. The non-adherent lymphocytes were cultured for 2 d at a cell density of 1.5×10^{6} cells ml^{-1} in Dulbecco's modified Eagle medium and 10% fetal calf serum. MCF-7, MDA-MB-157, MDA-MD-231, MDA-MB-330 and MDA-MB-134 are established human breast cancer cell lines. Values are means ±s.e.m. for 4 pairs of bone cultures.

*Significantly greater than 1.0, $P < 0.05$.

cancer cell lines were obtained from E. G. and G. Mason Research Institute.

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Selective expression of transduced or cloned DNA in minicells containing plasmid pKB280

DERIVATIVES of coliphage λ, carrying inserts of essentially any prokaryotic or eukaryotic DNA, can now be constructed using novel λ cloning vectors. Often, the reason for constructing these derivatives is to determine the polypeptides encoded by the inserted DNA. Phage expression can be analysed by infection of anucleate *Escherichia coli* minicells in which all host syntheses are absent[1]. However, analysis of the expression of the non-λ DNA insert in this system is complicated by the concurrent expression of λ DNA. Attempts to inhibit the expression of the λ genes by use of minicells from a λ lysogenic minicell-producing strain were unsuccessful, indicating that insufficient λ repressor was segregated into the minicells (Shaw, Epp, Pearson and J.N.R., in preparation). Recently a plasmid, pKB280, was constructed which carries the gene (*c*I) for the λ repressor protein and in which approximately 1% of the soluble protein of *E. coli* strains carrying this plasmid is λ repressor[2]. Minicells produced by a derivative of *E. coli* DS410 carrying plasmid pKB280 have been infected by transducing and cloning phages. We report here that only the non-λ DNA is expressed in these minicells.

E. coli DS410 (pKB280) was constructed by transformation of *E. coli* DS410 with purified plasmid DNA and selection for tetracycline resistance and immunity to λ. The plasmid would be expected to segregate into minicells, so that minicells produced by this strain should synthesise plasmid-encoded polypeptides. Figure 1 shows that minicells from *E. coli* DS410 (pKB280) synthesise large amounts of a polypeptide of molecular weight 26,000, presumably λ repressor, whose molecular weight from amino-acid sequence data is 26,228 (ref. 4). In addition, a further 24 polypeptides are detectable which, together with repressor, would require a total coding capacity of 10,700 base pairs of DNA. Plasmid pKB280 is approximately 6,800 base pairs in length[2,5]. It is clear, therefore, that the observed polypeptides exceed the coding capacity of the plasmid. As all the polypeptides synthesised are smaller than the repressor polypeptide, it seems likely that incomplete forms of repressor and/or proteolytic cleavage of the repressor occur in minicells.

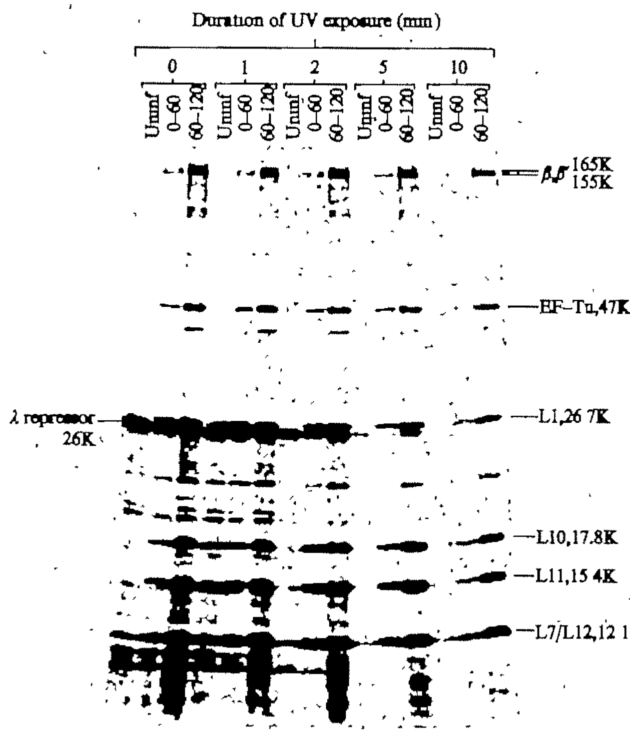

Fig. 1 Autoradiogram of the electrophoretic separation of the polypeptides synthesised in uninfected and λ*rif*^{d}18-infected minicells of *E. coli* DS410 (pKB280). Minicells were prepared as previously described and suspended (2×10^{10} per ml) in M9 minimal salts medium containing 20 μg ml^{-1} D-cycloserine[1,3]. The minicells were exposed to ultraviolet irradiation (290 μW cm^{-2}) for the indicated times. Aliquots of the infected minicells were infected (MOI $\hat{=}$ 5) with λ*rif*^{d}18 phage. Uninfected and infected minicells were incubated at 37 °C in the presence of ^{35}S-methionine assay medium[1,3]. The uninfected minicells and one aliquot of infected minicells (0–60) were allowed to incorporate the radioactivity for 1 h. A second aliquot of infected minicells was incubated in M9 glucose-salts minimal medium for 1 h at 37 °C before addition of ^{35}S-methionine assay medium These samples were then allowed to incorporate radioactivity for 1 h (60–120). Preparation of the radioactively labelled polypeptides synthesised in the minicells for electrophoresis, polyacrylamide gel electrophoresis through 14–20% polyacrylamide gradient gels, impregnation for fluorimetry and production of autoradiograms have been described previously[1,3]

Infection of the minicells by λ*rif*^{d}18 was used to test the function of the λ repressor in minicells. Phage λ*rif*^{d}18 carries the genes for β and β′ subunits of RNA polymerase, for EF-Tu and for the ribosomal proteins L1, L10, L11 and L7/L12 (refs 6–9). Infection results in the synthesis of only non-λ polypeptides encoded by λ*rif*^{d}18 in addition to the plasmid-encoded polypeptides (Fig. 1). No λ genes are expressed, indicating that the repressor inhibits the expression of the λ portion of λ*rif*^{d}18. The minicells were exposed to ultraviolet irradiation before infection, to destroy the synthesising ability of the plasmid (Fig. 1), and subsequent infection by λ*rif*^{d}18 resulted in the synthesis of only the non-λ polypeptides encoded by the phage genome.

Infection of the repressor-containing minicells by λwt phage results in the synthesis of reduced amounts of only two b2 region non-essential gene products (pEa47 and pEa59 in Fig. 2*a*). These two polypeptides have also been found to be synthesised in infected minicells in the absence of λ N protein (Shaw and J.N.R., unpublished observation). Fortuitously, the genes encoding these polypeptides are usually deleted in transducing or cloning phages, as in the phage λ*dgal*70/4 (ref. 10) shown in Fig. 2*b*. Infection by λ*dgal*70/4 of minicells containing repressor results in the synthesis of only the *gal* operon polypeptides.

There are basically two types of λ-cloning vectors. First, the insertion type in which only one restriction endonuclease site

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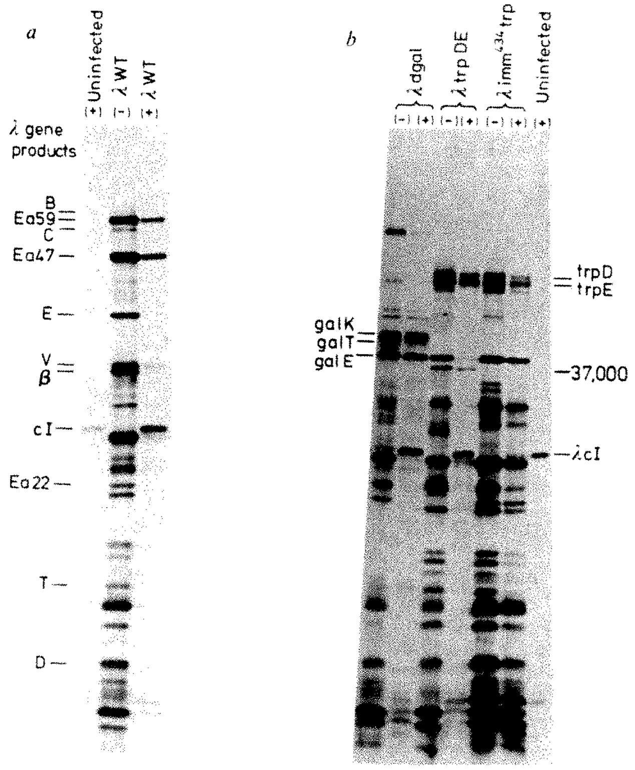
remains in the vector DNA and which is often located in the *c*I gene itself. Second, replacement vectors which have two restriction sites flanking a replaceable fragment of DNA[11]. Figure 2b shows the result of expression in minicells of an insertion vector (NM607 (ref. 11)) or replacement vector (NM596) which carry the same fragment of cloned DNA, namely the 4.7 megadalton fragment of DNA derived from plasmid VH151 (refs 12, 13) which encodes the *trpDEop* region of the *E. coli* chromosome. The *trpD* and *trpE* gene products can be readily identified from their published molecular weights[14] (Fig. 2*b*). Expression of the λ genes of the replacement vector is inhibited by the high concentration of λ repressor in the plasmid-containing minicells. Only the *trpD* and *trpE* gene products and a polypeptide of molecular weight 37,000 are synthesised in the presence of λ repressor. The 37,000 polypeptide may result from expression of the fusion of part of the *trpC* gene which is also carried on the cloned fragment[13] with the adjoining λ DNA. In contrast to the replacement vector, however, even in the presence of high concentrations of λ repressor, infection of minicells by the phage with the *trpDEop* fragment inserted in the *c*I gene results in the synthesis of polypeptides encoded by both the λ and the cloned DNA. This is exactly what would be expected, because the *c*I gene of this insertion vector, as found for many insertion vectors[11], is not the homologous λ *c*I gene but rather the *c*I gene of the lambdoid heteroimmune phage 434 (ref. 11). Expression is therefore insensitive to λ repressor. A plasmid carrying the immunity region of phage 434 has recently been isolated (A. Bailone and R. Devoret, personal communication). Construction of a minicell strain carrying this plasmid should provide a system in which DNA cloned in i^{434} phages can be selectively expressed.

Fig. 2 Comparison of the polypeptides synthesised in infected minicells in the presence and absence of λ repressor. Minicells were prepared from *E. coli* DS410 (−) and *E. coli* DS410 (pKB280) (+). The minicells were UV irradiated for 8 min (290 μW cm^{-2}) at a concentration of 2×10^{10} minicells per ml. *a*, Minicells infected by λwt ($\lambda c\mathrm{I}_{857}\mathrm{S}_{\mathrm{am}7}$); *b*, minicells infected by λ*dgal* 70/4 (ref. 10); λ replacement vector NM596, nin 5, carrying the *trpDEop* fragment of the *E. coli* chromosome[12,13] between *Eco*RI sites 2 and 3 of λ; λ insertion vector NM607, b_{538}, carrying the *trpDEop* fragment of the *E. coli* chromosome inserted in the *Eco*RI site located in the *imm*^{434} *c*I gene[11]. Infected and uninfected minicells were allowed to incorporate ^{35}S-methionine for 2 h at 37 °C in M9 minimal salts medium. Electrophoretic and other methods were as described in Fig. 1.



In conclusion, it seems possible to use ultraviolet-irradiated minicells produced by *E. coli* DS410 (pKB280) to express exclusively cloned or transduced DNA *in vivo*. The expression requires that the fragment of DNA of interest carries a promoter which is insensitive to λ repressor. Expression of fragments of DNA which do not carry promoters may still be analysed in minicells without λ repressor; however, λ expression will also occur[1]. Comparison of the polypeptides synthesised in minicells containing or lacking repressor infected by vector phage with and without cloned or transduced DNA should enable the following questions to be answered. (1) Are genes on the fragment of DNA expressed in *E. coli*? (2) Does the fragment of DNA carry a promoter which functions in *E. coli*? (3) If (1) is positive, then how many and what are the molecular weights of the encoded polypeptides (or mRNAs), and if (2) is positive, can the expression be affected by the experimental conditions, such as an inducing compared with a non-inducing medium?

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Note added in proof: Minicells from *E. coli* DS410 carrying the plasmid pGY101 (from R. Devoret) which confers immunity to i^{434} phages have been found to function as predicted and permit the selective expression of non-λ DNA contained in i^{434} vector genomes.

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Assembly of stratum corneum basic protein and keratin filaments in macrofibrils

THE stratum corneum is the most superficial aspect of mammalian epidermis and consists of several layers of fully differentiated, dead cells filled with an insoluble protein complex called keratin. Early ultrastructural studies of the stratum corneum cells revealed a 'keratin pattern' of light-staining filaments 70–80 Å in diameter embedded in a dark-staining osmiophilic amorphous matrix[1]. The filaments are synthesised in the inner living cell layers and are variously termed tonofilaments, α-keratin or, more accurately, keratin filaments. The subunits of the filaments of both the living cells and stratum corneum have been extracted with dissociating reagents from bovine[2–4], rat[5–6], mouse[7], and human[8] epidermis. In appropriate conditions, these subunits will polymerise *in vitro* into native-type keratin

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filaments that are uniformally 70–80 Å in diameter[7,9]. In contrast, the amorphous matrix has not been positively identified at the biochemical level, although it was recently suggested that a stratum corneum basic protein (SCBP) rich in histidine has properties consistent with a possible function as an interfilamentous matrix substance[10]. We show here that highly ordered structures reminiscent of the keratin pattern in the intact stratum corneum form when the SCBP and epidermal keratin filaments are combined *in vitro*.

SCBP was purified from newborn rat epidermis as previously described[10]. The keratin filament subunits were isolated from the same source by a modification of previous techniques[3,5]. Other fibrous proteins were also combined with SCBP in the aggregation experiments. These included: keratin filaments assembled *in vitro* from the subunits extracted from mouse[7], human and bovine[9] epidermis; β-keratin filaments polymerised *in vitro* from the *S*-carboxymethyl derivatives of chicken feather[11]; mouse skin collagen, types I and IV (ref. 12); F-actin isolated from mouse skeletal muscle and mouse epidermal cells grown in culture[13].

The rat epidermal keratin filaments outlined by negative-staining with uranyl acetate are apparently identical to those of other species[7,9]. They are long rods uniformly 70–80 Å in diameter, but in places display a distinct electron-dense central line suggestive of a core (Fig. 1*a*).

When SCBP was combined with these filaments in ratios as low as 0.1 : 1 (by weight), there was an immediate increase in turbidity indicating association of the proteins. At ratios of 0.2 : 1 or greater, easily observable fibrous-like aggregates precipitated from suspension. The amount of aggregate increased with increasing ratios of SCBP to filaments up to about 2 : 1. Under the electron microscope, these aggregates were composed of large numbers of orientated keratin filaments (Fig. 1*b*,*c*). Bundles of filaments in differentiating keratinocytes are termed macrofibrils[16], a term which might also accurately describe these aggregates formed *in vitro*. When formed at low SCBP-to-filament ratios, filaments in the macrofibrils assembled in either a loose or tight organisation but when formed at higher ratios, more compact macrofibrils generally resulted in which filaments were highly aligned and tightly packed. The macrofibrils formed within seconds of mixing and were stable for several days at room temperature and indefinitely at 4°C.

Samples of the macrofibrils were fixed, embedded, sectioned and observed in both longitudinal and cross section. In longitudinal section, lightly stained filaments 70–80 Å in diameter were aligned in close apposition and were separated at an approximately uniform distance by dark-staining material (Fig. 2*b*). In cross section, darkly stained dots were observed surrounded by light haloes. These structures corresponded to the central dense core and the electron-lucent portions of the filament. The diameter of the light halo is identical to the diameter of the filaments seen in longitudinal sections. The filaments, in turn, were outlined by darker-staining material which filled the interfilamentous interstices (Fig. 2*c*). This pattern closely resembles the keratin pattern of certain stratum corneum cells.

Two types of control experiments were performed. Cytochrome *c* and a histone preparation (calf thymus, type II, Sigma) were each tested as alternate basic proteins. When mixed with rat keratin filaments, there was a slight increase in turbidity indicating association of the basic proteins with the anionic filaments, but no macrofibrils formed. The individual filaments were coated with an amorphous layer of protein and, as a result, seemed irregular in diameter. Other filament preparations were also tested. β-Keratin filaments from chicken feather, mouse dermal collagen types I and IV and F-actin from mouse skeletal

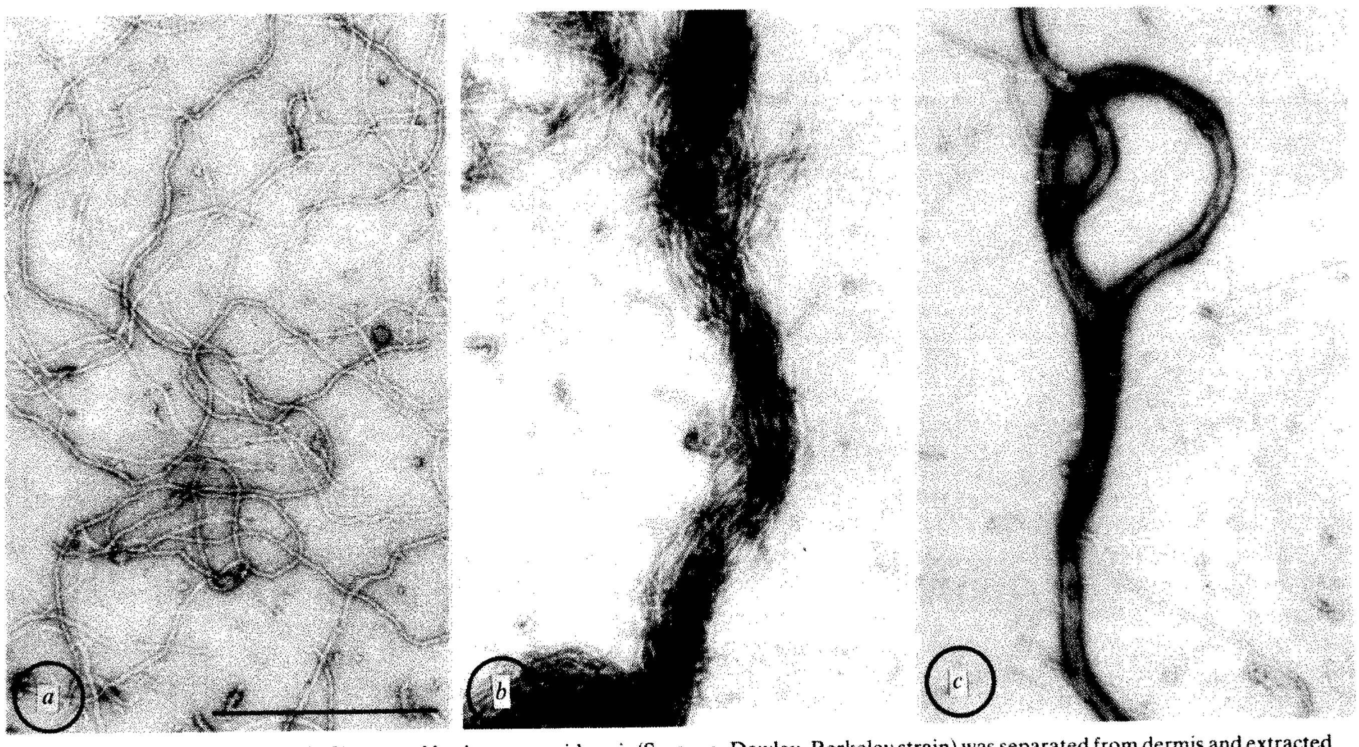

Fig. 1 *a*, Rat epidermal keratin filaments. Newborn rat epidermis (Sprague–Dawley, Berkeley strain) was separated from dermis and extracted first with 1 M potassium phosphate (*p*H 7.0) to remove keratohyalin[14], then with 4 M urea in 0.05 M phosphate (*p*H 7.0) for 3 h at 23 °C and with 8 M urea in 0.1 M Tris-HCl (*p*H 8.5) containing 0.1 M 2-mercaptoethanol for 16 h at 23 °C. All extraction buffers contained the protease inhibitor phenylmethylsulphonyl fluoride. The 4 M urea extract was used to prepare SCBP[10], and the 8 M urea extract was the source of the keratin filament subunits. After further purification by DEAE-cellulose chromatography[15], these subunits (1 mg ml^{-1}) were dialysed against 5 mM Tris-HCl (*p*H 7.6) containing 1 mM dithiothreitol or 25 mM 2-mercaptoethanol for 16 h at 23 °C during which time filaments formed in suspension. For negative staining, filament suspensions were diluted to approximately 0.1 mg ml^{-1}, placed drop by drop onto parlodion and carbon-coated grids, stained with 1% uranyl acetate, dried and examined in a Philips 201 transmission electron microscope (TEM). *b* and *c*, recombination of rat epidermal keratin filaments with SCBP. SCBP and filaments were equilibrated at 1 mg ml^{-1} in 5 mM Tris-HCl (*p*H 7.6). Samples of the two solutions were mixed at an SCBP-to-filament ratio of approximately 0.25 : 1 (by weight), allowed to stand for 5 min, placed drop by drop on grids, negatively stained with uranyl acetate and examined by TEM. *b*, Loose rope-like aggregates of aligned filaments; *c*, compact macrofibrils with highly aligned filaments. Scale bar, 0.5 μm.

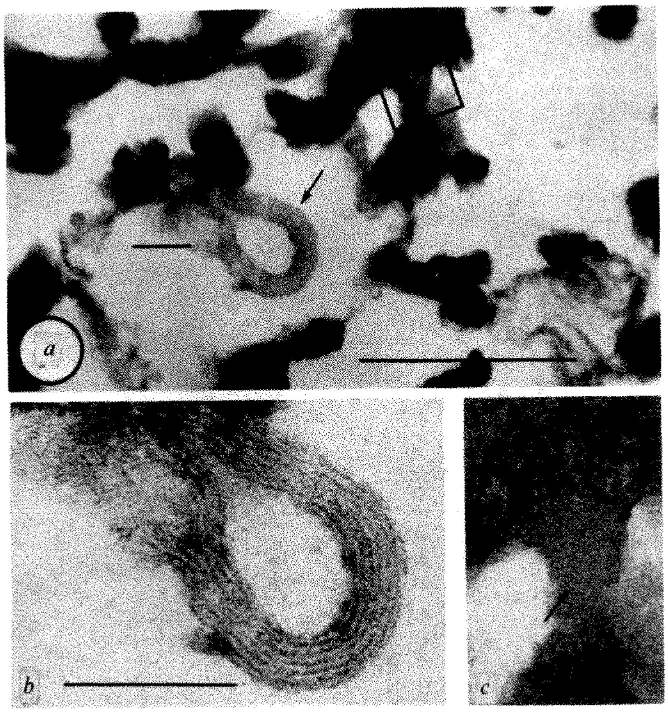

Fig. 2 Ultrastructure of fixed, embedded macrofibrils formed *in vitro* at a ratio of SCBP to filaments of approximately 4:1. Macrofibrils were withdrawn from the tube, fixed for 1 h in half-strength Karnovsky fixative[17], dehydrated through a graded series of alcohols, and embedded in Epon[18]. *a*, Filaments seem to be combined with sparse (arrow) and abundant (box) quantities of SCBP. Scale bar, 0.5 μm. *b*, Aligned filaments in longitudinal section sparsely coated with SCBP. Scale bar, 0.2 μm. *c*, Cross-sectional view of filaments in a large amount of SCBP. Arrows indicate the black dot of the central dense line within the filament. Same magnification as (*b*).

or cultured mouse epidermal cells did not aggregate into fibres or form macrofibrils when mixed with SCBP. In each case, an increase in turbidity was seen on mixing and the fibrous protein was coated in an apparently random manner by the SCBP.

Thus the formation of macrofibrils *in vitro* is specific for SCBP and epidermal α-keratin filaments. This specificity, the highly orientated nature of these structures, and their close resemblance to the structure seen in intact stratum corneum, lead us to conclude that the SCBP may be an interfilamentous or matrix protein of epidermal keratin.

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Actin microcrystals and tubes formed in the presence of gadolinium ions

SKELETAL muscle actin can be isolated either as a globular monomer, G-actin, or as a filamentous polymer, F-actin[1,2]. The transition of G to F-actin is achieved by the addition of salts of monovalent or divalent cations. In the presence of higher concentrations of divalent cations F-actin filaments further

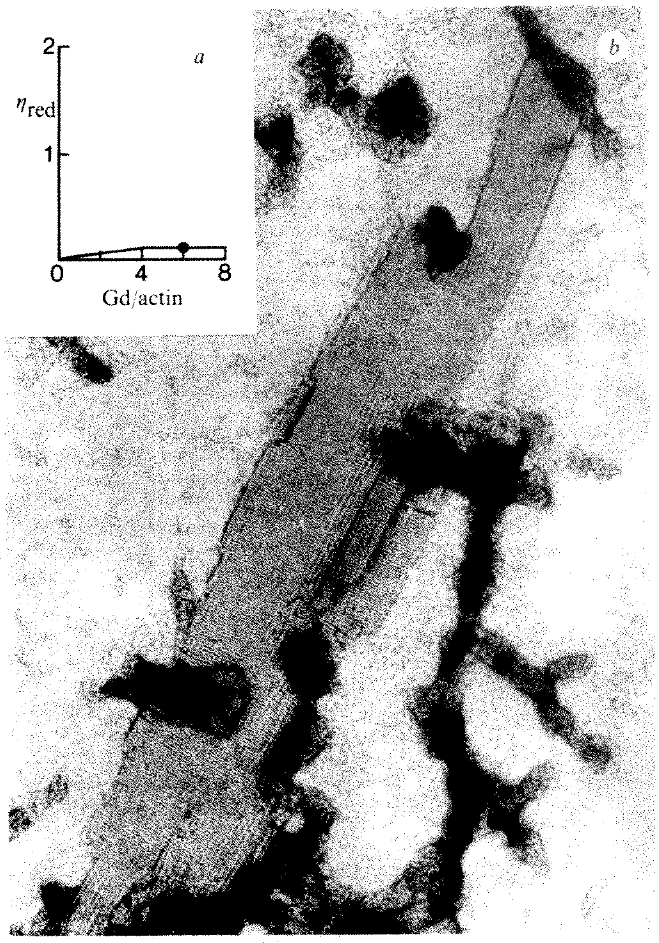


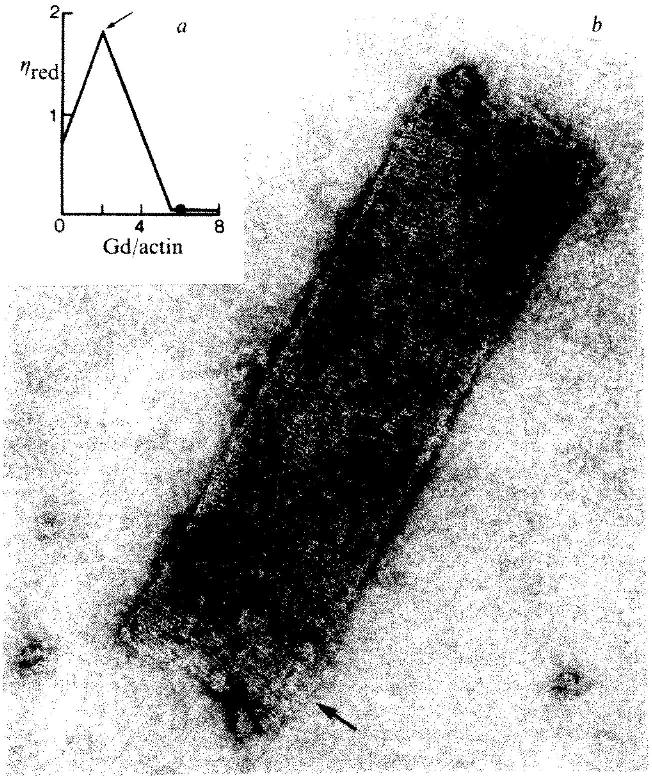
Fig. 1 *a*, Effect of Gd III on the reduced viscosity of actin in the absence of KCl. Reaction conditions are: 0.2 mM ATP; 0.5 mM β-mercaptoethanol; 2 mM PIPES at *p*H 6.95; 25 °C; $GdCl_3$ added at the molar ratios indicated on the abscissa. As Gd III binds to ATP the amount stated was that in excess of the ATP present[8,9]. 1.15 mg ml^{-1} G-actin was prepared from rabbit skeletal muscle by the method of Spudich and Watt[10]. Steady state $\eta_{red} = [(ts/tw) - 1/C$ ml mg^{-1}: where *ts* is the flow time (s) of the sample, *tw* is the flow time of the solvent (31.5 s) and *C* is the G-actin concentration (mg ml^{-1}) as determined by the Lowry method[11]. *b*, Actin microcrystal formed with Gd III in the conditions specified by the black dot in (*a*). The overall dimensions of the polymer are about 1 μm × 0.2 μm. In some experiments microcrystals were formed by adding Gd III slowly by dialysis. Specimens were supported on thin carbon-coated copper grids and negatively stained with 1% uranyl acetate. Magnification was calibrated with the known 14.4-nm repeat of thick filaments of the oyster adductor muscle[12]. ×143,000

aggregate into paracrystalline arrays[3–6]. We present here evidence for two new types of ordered actin assembly formed in the presence of trivalent lanthanide cations, which are not composed of F-actin filaments.

Recently we have studied the effects of trivalent lanthanides on the viscosity of G-actin in the absence[7] and presence[8] of 0.1 M KCl. These data suggest that amounts of lanthanide ions greater than 5.5 mols per mol of actin cause actin to aggregate into a form different from F-actin. We have now investigated in the electron microscope the aggregates induced by the trivalent lanthanide gadolinium (Gd III). Figure 1*b* shows that without added KCl, Gd III ions cause G-actin to assemble into sheet-like structures which we will refer to as actin microcrystals. These polymers are formed at molar ratios higher than 4 mols Gd III per mol of G-actin. Their dimensions vary but they are usually not longer than 1 μm and not wider than 0.3 μm. There are often sharp steps in the staining density across the structure which suggests that they consist of more than one layer. The microcrystal in Fig. 1*b* is at least three layers thick. They have a prominent axial period of approximately 3.3 nm which is best observed when micrographs are viewed obliquely from the side. A less clear transverse repeat of about 6.0 nm is occasionally seen when the structures are viewed obliquely along their length but this was difficult to measure accurately. Microcrystals differ markedly in appearance from actin paracrystals. In particular they do not show the 36-nm axial repeat characteristic of the long pitched helical repeat of F-actin[3,4]. Some less well-ordered rope-like aggregates of actin can also be seen in Fig. 1*b*, but no individual filaments of F-actin were present in these preparations.

G-actin forms different polymers when incubation with Gd III ions is followed by the addition of 0.1 M KCl. Figure 2*a* summarises our previously reported data on the changes in viscosity in these conditions[7,8]. Figure 2*b* shows an example of the polymers formed in the presence of KCl at a molar ratio of 6 mols or more of Gd III per mol actin. The length of the polymers is variable from ~0.3 to ~1.5 μm but each polymer has a uniform width of about 200 nm ± 10 nm. In each polymer two sets of diagonal striations spaced about 5.5 nm apart can be seen intersecting one another at equal and opposite angles of approximately 80° with respect to the sides of the structure. This is seen more clearly when the micrograph is viewed at an oblique angle. Occasionally at the ends of the polymer only one set of striations is observed and here the 5.5-nm repeat is distinctly seen. These observations suggest that the main part of the polymer is a flattened tube with the two sets of striations coming from strands that form the front and back of the polymer. We therefore propose to call these structures actin tubes. As in the microcrystals, no 36-nm repeat and no F-actin filaments can be seen in these preparations. Rope-like aggregates which have a similar appearance to the extra material seen with microcrystals are sometimes seen associated with actin tubes. This extra material may be associated with the formation of both structures.

Fig. 2 *a*, Effect of Gd III on the reduced viscosity of actin in the presence of KCl. Reaction conditions are the same as described in Fig. 1*a* except that following a 5 min incubation of G-actin with Gd III, KCl was added to give a final concentration of 0.1 M. *b*, An actin tube formed with Gd III in conditions specified by the black dot in 2*a*. The arrow indicates an end of a tube where only one side of the structure can be seen. Negatively stained with 1% uranyl acetate. ×134,000.



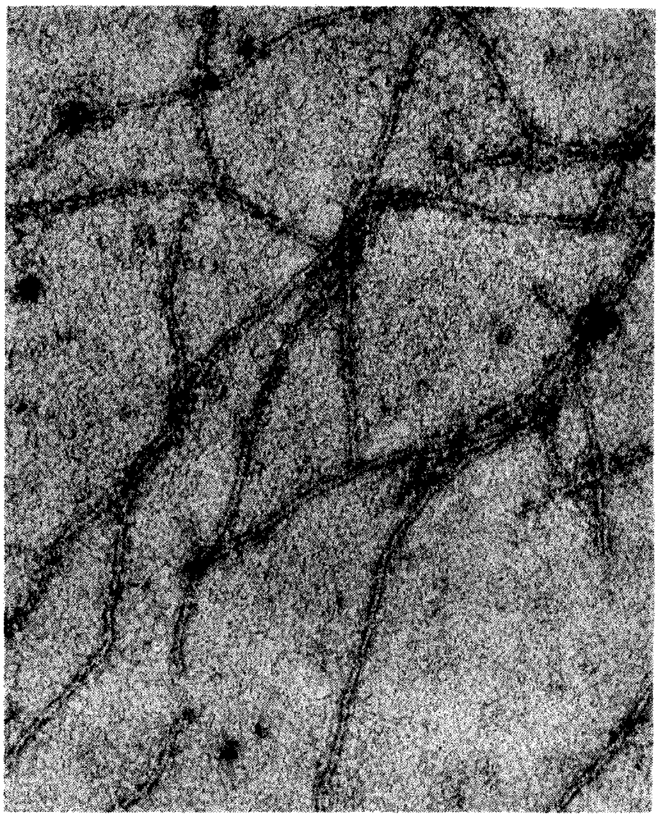


Fig. 3 Network of F-actin formed at the peak of the reduced viscosity in the conditions indicated by the arrow in Fig. 2*a*. No actin tubes were observed in these conditions stained with 1% uranyl acetate. ×126,000.

We have shown (Fig. 2) that in the presence of 0.1 M KCl actin tubes are formed with 6 mols or more of Gd III per mol G-actin. No F-actin is formed as judged either by viscometry or by electron microscopy. However, with less than 5.5 mols Gd III per mol G-actin, addition of 0.1 M KCl causes a large increase in the observed viscosity, suggesting that polymerisation into F-actin had occurred. Samples examined at the peak of the viscosity in the electron microscope (Fig. 3) show that the actin has polymerised into F-actin filaments which have aggregated into a loose meshwork. In a few places we can observe a side-by-side arrangement of the filaments. F-actin were observed over the range 0–5.5 mols Gd III per mol actin. Thus the lanthanide ions do not inhibit the formation of F-actin until a certain critical molar ratio is reached.

The actin used in this study migrated as a single band on SDS gel electrophoresis. The formation of actin microcrystals and actin tubes is completely reversible when the Gd III concentration is lowered. There is therefore little doubt that these structures are composed entirely of actin and that their formation is due to the presence of the lanthanide ions. Furthermore, G-actin after incubation with Gd III followed by removal of the lanthanide will polymerise into F-actin[8]. It is evident that the actin has not been denatured by the lanthanide ions. The absence of F-actin formation together with the observation that neither the microcrystals nor the actin tubes possess the 36-nm axial repeat characteristic of the long helical pitch of F-actin shows that these structures are not composed of F-actin and represent some previously undescribed form. Further structural studies are in progress (M. J. D. and C. G. dos R., in preparation). These modes of actin assembly should provide new insights into the size, shape and molecular interactions of actin subunits.

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Asymmetry in the primary structure of undegraded phytochrome

PHYTOCHROME is the photoreceptor for many light-mediated developmental responses in plants[1]. This chromoprotein, considered a dimer of apparently identical 120,000-dalton subunits (undegraded phytochrome)[2,3], has often been purified from oats as a monomeric 60,000-dalton degradation product (degraded phytochrome)[2,3] because of its susceptibility to proteases present in crude extracts[3]. Degraded phytochrome is relatively resistant to further proteolysis and has almost the same photochemical properties as undegraded phytochrome[2]. Immunochemical evidence[4] indicates that undegraded phytochrome is asymmetric, possessing extensive primary structure not found in degraded phytochrome. In contrast, recent peptide-map data, obtained by a fluorescence procedure, have led to the conclusion that undegraded phytochrome is symmetric, being composed of two nearly identical halves, each half being equivalent to the 60,000-dalton, photoreversible degradation product[5]. We present here autoradiographic tryptic digest maps of iodinated degraded and undegraded phytochrome from etiolated oat (*Avena sativa* L., cv. Garry) seedlings that offer additional evidence concerning asymmetry in the primary structure of undegraded phytochrome.

Two tryptic digest maps of degraded and five maps of undegraded phytochrome were prepared from the same number of different preparations using phytochrome bands cut from SDS–polyacrylamide gels (Fig. 1). The map of degraded phytochrome (Fig. 2*a*) contains all but one of the spots (unlabelled arrow) found on the map of undegraded phytochrome (Fig. 2*b*), which contains nine additional spots of comparable intensity (unlabelled arrows). Replicate maps (not shown) gave the same patterns as in Fig. 2. Unmarked spots seen on one map but not the other are revealed by longer exposures of the same maps and therefore are not unique. The existence of a unique spot on the degraded phytochrome map is reasonable as the endoproteases that produce degraded phytochrome may not have the same specificity as trypsin. At least one terminus of the degraded chromopeptide would then have been cleaved between residues that are recognised by trypsin. The peptide subsequently derived by trypsin degradation would have only part of the primary structure found in the analogous peptide in phytochrome that had not been degraded by endogenous proteases. This peptide could also account for one of the additional spots on the undegraded phytochrome map. The remaining eight additional spots represent peptides not found in degraded phytochrome. Even if our interpretation of which spots are homologous (lettered arrows) is in error, the number of spots on the undegraded phytochrome map that cannot be accounted for on the degraded phytochrome map would still be a minimum of eight. As the number of spots on the map of undegraded phytochrome is almost twice that on the degraded phytochrome map, undegraded phytochrome is apparently composed of only one copy of the degraded molecule and an approximately equal amount of unrelated primary structure.

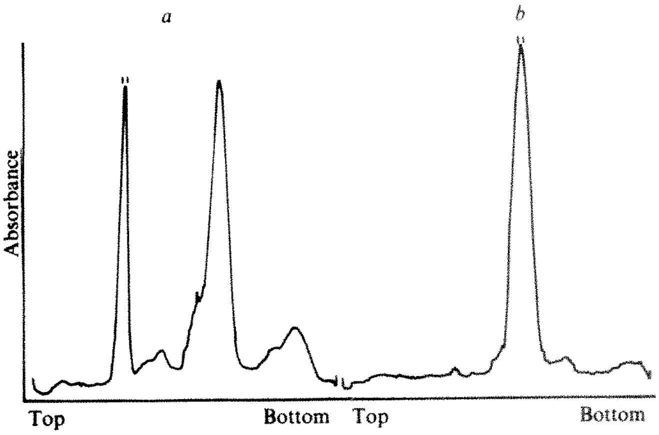

Fig. 1 Absorbance scans of 5 mm diameter, cylindrical SDS–polyacrylamide gels of *a*, an immunoprecipitate of $\sim 10\ \mu g$ of undegraded phytochrome, with the 120,000-dalton phytochrome subunit (left) and the 50,000-dalton heavy and 25,000-dalton light chains of immunoglobulin G, and *b*, $\sim 10\ \mu g$ of purified 60,000-dalton degraded phytochrome. The portion of each phytochrome band (central 1 mm) cut out for analysis is indicated by the hash marks. Undegraded phytochrome was partially purified from dark-grown oat seedlings through the first ammonium sulphate fractionation as described previously[6], and then precipitated with specific antiphytochrome serum. Degraded phytochrome was purified from the same oat cultivar using the method described by Rice *et al.*[7], except that all buffers before the Bio-Gel P-150 column contained 14 mM 2-mercaptoethanol, and a linear salt gradient elution from brushite was performed before Bio-Gel P-150 chromatography. Both phytochrome preparations were denatured by heating in 1% (w/v) SDS and 0.79 M 2-mercaptoethanol, were electrophoresed through 5% (w/v) polyacrylamide gels with 0.14% (w/v) *N-N'*-methylenebisacrylamide for 5h at 8 mA per gel, as described by Weber and Osborn[8], and were stained for protein using Coomassie blue. Mobilities of proteins were compared to standards for size determination (see Fig. 3 in ref. 2).

It has been argued that 40–50 peptides represents the upper limit of resolution of a two-dimensional peptide map[10]. As tryptic digestion of even degraded phytochrome yields at least 55–60 peptides[5], the selectivity of the method used here in

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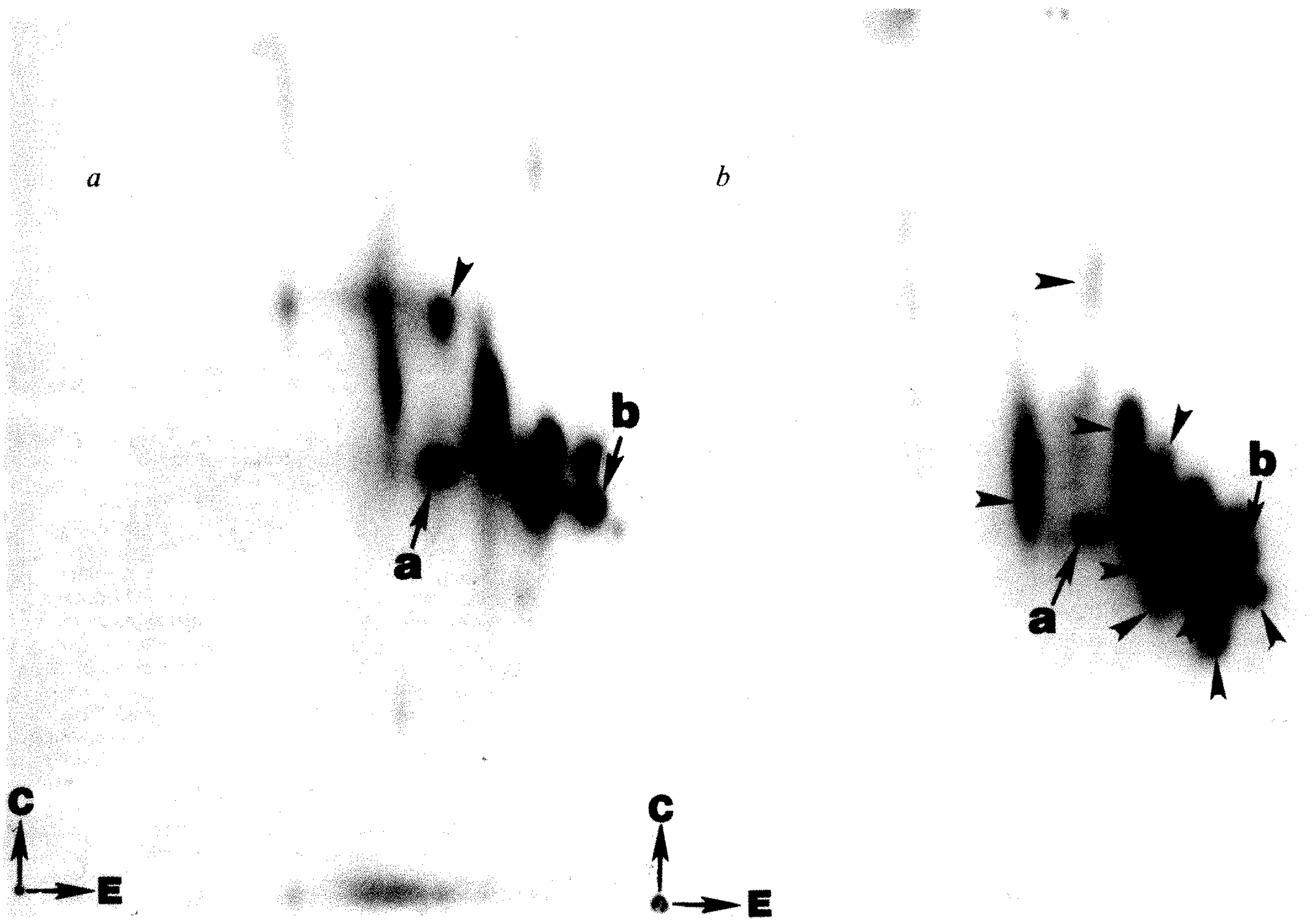

Fig. 2 Autoradiographs of tryptic digest maps of *a*, degraded and *b*, undegraded phytochrome prepared in a single experiment. Lettered arrows indicate corresponding spots; unlettered arrows unique spots. Two dimensional tryptic fingerprinting of ^{125}I-labelled polypeptides was performed essentially as described by Elder *et al.*[9] Each 1-mm thick gel slice (see Fig. 1) was placed in a siliconised test tube, washed extensively with 25% (v/v) isopropanol and then with 10% (v/v) methanol and dried by vacuum desiccation. The following components were added sequentially to each dried gel slice: 20 μl 0.5 M sodium phosphate buffer (*p*H 7.5), 300 μCi sodium ^{125}I (NEN, carrier free, 17 Ci mg^{-1}) and 5 μl 1 mg ml^{-1} chloramine T (Sigma). The gel slices were allowed to absorb the liquid for 30–45 min, and then 1 ml of 1 mg ml^{-1} sodium bisulphite was added to terminate the reactions. After 15 min the bisulphite solution was removed. The slices were placed into individual nylon pouches and allowed to dialyse at least 24 h against 4 one-litre changes of 10% (v/v) methanol. Following dialysis each gel slice was removed from its pouch with a spatula, placed in a siliconised tube and dried by vacuum desiccation. To each tube 1 ml of 50 μg ml^{-1} TPCK-trypsin (Worthington) in 50 mM ammonium bicarbonate was added. The gel slices were incubated for 24 h at 37 °C after which each supernatant was removed and lyophilised. Each trypsin-digested sample was dissolved in 20 μl of acetic acid, formic acid and water (15 : 5 : 80), and then 5–10 μl of the samples were spotted on 20 × 20 cm cellulose-coated thin layer plates (Eastman, 13255 cellulose, without fluorescent indicator) for electrophoresis. Electrophoresis was carried out for 1 h at 1 kV in a Savant flat bed electrophoresis apparatus maintained at 2–4 °C. After electrophoresis the plates were dried and the peptides chromatographed in a second dimension using butanol, pyridine, acetic acid and water (32.5 : 25 : 5 : 20). Following chromatography the plates were dried and wrapped in cellophane. Plates were placed over X-Omat R X-ray film (Eastman) so that peptides could be located by autoradiography. Both plates were photographed on a single roll of Kodak Panatomic X film and were printed on paper of identical contrast. Directions of electrophoresis (E) and chromatography (C) are indicated at the origin. Longer exposures of the maps (not shown) indicate that spots on a map that are unmarked (for example, Fig. 2*a*, intense spot immediately to the left of unmarked arrow and numerous faint spots around main pattern) are also found on the alternate map and therefore are not unique.

visualising primarily only tyrosine-containing peptides, though a potential drawback, is advantageous for our purposes. Residues other than tyrosine are also labelled by this method, but at a lower rate. In addition, the rate of deiodination of these other residues is rapid enough to make it very unlikely that any major spot is due to iodination of a residue other than tyrosine[11]. As the samples were handled identically, any potential labelling of other amino acids would be identical for both maps.

Amino acid analyses indicate that degraded phytochrome contains 10–18 tyrosyl residues and undegraded about 26 residues[2,5]. These are sufficient tyrosines to permit critical comparison of the two maps, but few enough to minimise apparent coincidence of similarly migrating, but non-homologous, peptides. The number of spots on the undegraded phytochrome map is smaller than the expected maximum of 26, probably because some peptides contain multiple tyrosines. The possibility that a peptide is seen in only one map because of the absence of a labelled tyrosine in one case but not the other is precluded because both maps are derived from the same gene products and because these products are labelled after denaturation. Labelling of each sample seems to be complete as the map of degraded phytochrome is completely contained in the map of undegraded phytochrome with the exception of the spot referred to above. Peptides arising from the self-digestion of trypsin[12] are not visualised on the autoradiographs of our maps because trypsin is not added until after residual ^{125}I and chloramine T are removed. Coincidence of a serum protein with the 120,000-dalton phytochrome band is also not a problem as crude serum has no band at this position. Maps prepared from gel slices lacking protein gave totally blank autoradiographs.

The conclusion derived from immunochemical evidence[4] is the same as that derived from the peptide maps reported here.

Limited proteolysis of undegraded phytochrome yields at least two distinct peptides recognised by antiserum against undegraded but not degraded phytochrome. One of these peptides is ~90,000 daltons as estimated by gel exclusion chromatography. This size, which is probably an overestimate due to non-globular shape, indicates that the peptide, which does not contain a detectable chromophore, represents about one-half of the 120,000-dalton monomer of undegraded phytochrome. As each of the large variety of immunoglobulin Gs in the antiserum against degraded phytochrome recognises a different section of primary structure[13], it is very unlikely that this '90,000-dalton' peptide has any appreciable primary structure in common with degraded phytochrome.

The data presented here, together with the immunochemical data published earlier[4], support the same conclusion: that the 120,000-dalton, undegraded phytochrome subunit contains a single copy of the 60,000-dalton, degraded phytochrome monomer and approximately 60,000 daltons of unrelated primary structure. This conclusion conflicts with the earlier interpretation by Stoker *et al.*[5] of peptide maps (photographs of which were not presented) that visualised all products of tryptic digestion, rather than the limited number examined here.

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C-terminal side of α-helix is more stable than N-terminal side

THE stability of the polypeptide chain of the α-helix has been extensively studied by both experimental and theoretical examination of the transition from the α-helical to random coil conformation of different homopolypeptides[1,2]. In these studies, the α-helix has been treated symmetrically along the helical axis. Amino acid residues located at the *n*th position from the N terminus and the C terminus of the α-helix in a homopolypeptide are assumed to behave in the same manner. However, the stability of the α-helix in the N-terminal region must be different from that in the C-terminal region, due to some extent to the asymmetrical nature of the peptide bond. It has not previously been known whether or not the influence of a polypeptide in random conformation is independent of the side of the α-helix to which the polypeptide is attached. We report here the first experimental evidence for such asymmetrical behaviour.

To detect asymmetry in the α-helix, we prepared block co-polypeptides such that their amino acid compositions were the same but their sequences were reversed with respect to each other; asymmetrical differences in a homopolypeptide are not distinguishable. As previously described[3], we synthesised two kinds of block co-polypeptides, $(\text{L-Glu})_{20}(\text{L-Ala})_{20}\text{Phe}$ (designated EAF) and $(\text{L-Ala})_{20}(\text{L-Glu})_{20}\text{Phe}$ (designated AEF) by a fragment-peptide-coupling technique on a solid support using $(\text{BOC-L-Glu})_4$ and $(\text{BOC-L-Ala})_2$. We determined the difference between the α-helical content of each block co-polypeptide in aqueous solution at the same *p*H, temperature and salt concentration as described below.

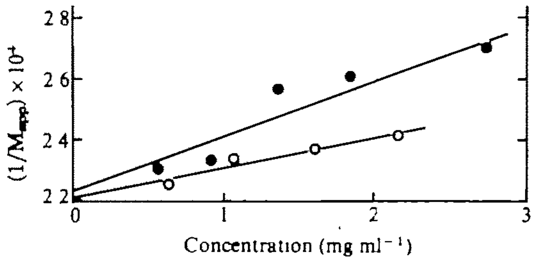

Fig. 1 Results of sedimentation equilibrium experiments for EAF (●) and AEF (○). The experiments were carried out with a Spinco model E analytical ultracentrifuge using the interference optics at 25 °C at 29,500 r.p.m. for 24 h. The block co-polypeptides were dissolved in 0.1 M KF, 20 mM Na-phosphate buffer, *p*H 8.0 (KF was selected as a neutral salt with a low extinction to 190 nm to measure CD spectra). A partial specific volume of block polypeptides, $\bar{v}$, was taken as 0.63, which is the average weight of partial specific volumes of potassium glutamate (0 57) (refs 12, 13) and alanine (0.74) (ref. 14). The molecular weights (MW) of both polypeptides extrapolated to infinite dilution by the least-squares method were 4,500 M_{app}, apparent molecular weight.

Care was taken to obtain uniform block co-polypeptides. The polypeptides, fractionated by repeated gel filtration on Sephadex G-50, had amino acid compositions of Ala = 21.4, Glu = 21.7, against Phe at the C-terminal end (used as the internal standard) for EAF, and Ala = 19.9, Glu = 20.8 for AEF, in agreement with the calculated values. The presence of any aggregations or micelles could result in artificial differences in the α-helical contents of both block co-polypeptides; micelle formation might occur rather easily for polypeptides containing polyalanine. The materials were monodisperse in solution, as rechromatography on Sephadex G-50 gave only a single peak. This observation was supported by the sedimentation equilibrium experiments shown in Fig. 1. Both polypeptides had the same molecular weight of 4,500, in good agreement with the calculated values from the amino acid compositions. The finding that the slopes against concentration (that is, the second virial coefficients) are different—greater for EAF than for AEF—suggested that the conformations differ from each other in solution. As micelle formation seemed likely to be the most serious problem in this experiment, the circular dichroic (CD) measurements were carried out at various concentrations in the range 0.003–3 mg ml⁻¹. No concentration effect was observed and so further measurements were made at a concentration of 0.1 mg ml⁻¹.

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Figure 2 shows the CD spectra of EAF at *p*H 5.22, 5.56, and 8.53 at 25 °C. The typical CD curve of the α-helix at *p*H 5.22 has two minima, at 223 nm and 208 nm, and one maximum, at 191 nm; it changes with the increase in *p*H, indicating that the ionisation of carboxyl groups in L-Glu residues causes the collapse of the α-helix into the random coil conformation. The presence of the isodichroic point at 204 nm implies that the transition occurs between two states, α-helix and random coil. The α-helical spectrum represented by the minimum at 223 nm still occurs at *p*H 8.53; this may be attributed to the polyalanyl block in the material, for there is no ionisable group in the alanine side chain, whereas the polyglutamyl block takes a random coil conformation at this *p*H. We examined whether or not the CD spectra of AEF are coincident with those of EAF.

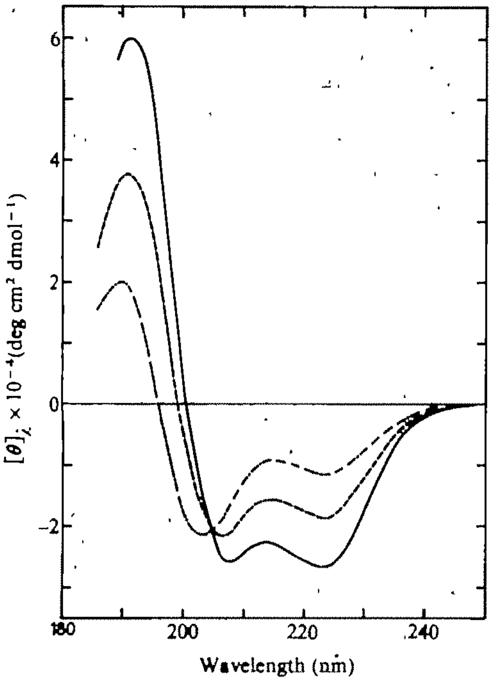

Fig. 2 Circular dichroic spectra for EAF at 25 °C. Solid line, *p*H 5.22; dashed line, *p*H 5 56; dashed-dotted line, *p*H 8.53. CD measurements were carried out with a JASCO J-20 spectropolarimeter using a cell of 1 mm path length. The *p*H of solutions (in 0.1 M KF) was adjusted by the addition of a small amount of KOH or $HClO_4$ to the desired values. Concentrations of polypeptides were 0.1 mg ml^{-1}.

Plots of ellipticities at 223 nm, $[\theta]_{223}$ (a measure of α-helical content), for EAF and AEF are shown in Fig. 3 as a function of *p*H. Apparently, $[\theta]_{223}$ increases with increase in *p*H and the transition *p*H of EAF is higher (*p*H 5.4) than that of AEF (below *p*H 5.0). The values of $[\theta]_{223}$ become constant above *p*H 6.5, but are always lower for AEF than for EAF. These results indicate that EAF has a higher α-helical content (32% calculated on the basis that $[\theta]_{223} = -37{,}000$ deg cm^2 dmol^{-1} for the full α-helix, and 900 for the random coil[4–6]) than AEF (16%) in the same conditions. As the block co-polypeptides are monodisperse in solution (Fig. 1), the stability of the α-helix of the polyalanyl portion must be affected differently by the conformation of the polyglutamyl block and depends on which side the block is connected, the polyalanine helix being more unstable when the charged polyglutamyl block is attached to the C terminus of the helix. Experiments on thermal denaturation of the same materials were consistent with these results, and will be reported in detail elsewhere.

The difference might originate from the Phe residue added to the C terminus as the internal standard, through hydrophobic stabilisation of the α-helix. However, a single additional residue is not sufficient to account for the observed difference of 16% in the helical content. If this were the reason, one extra residue at the C terminus should exert a stabilising effect over 6 to 7 residues in the α-helix. Another possible cause for the asymmetry might be some contribution from the electrostatic interaction of the negative charges on Glu residues with the helix dipole or with peptide dipoles. As the electrostatic interaction in aqueous solution may be weakened by the addition of salts, we examined the effect of salts on the results. The difference observed even at 0.5 M concentration of salts show that the electrostatic effects are not enough to explain the asymmetry, although further detailed examination is required. Therefore, we may conclude that the difference is based on an intrinsic property of the α-helix.

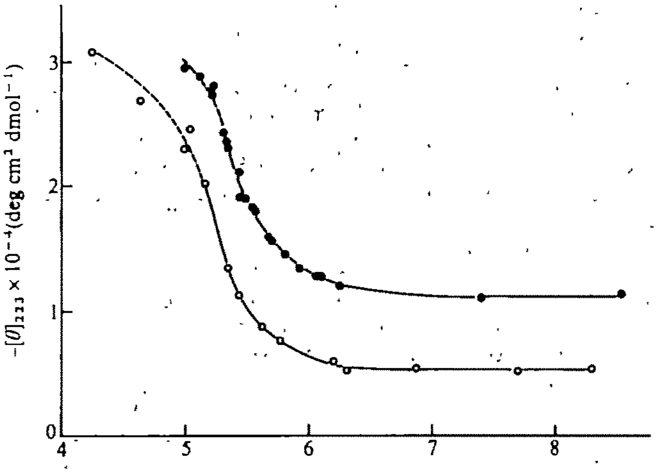

Fig. 3 Ellipticities at 223 nm, $[\theta]_{223}$, plotted against *p*H for EAF (●) and AEF (○) at 25 °C in 0.1 M KF. The dashed lines represent the region where precipitates appeared. The solutions in this region became turbid with standing, so that accurate measurements were impossible below *p*H 5.0.

The presence of the asymmetry reported in this study was suggested by simple theoretical calculations on the α-helical conformation[7,8]. Also, conformational analyses on three-dimensional structures of proteins demonstrate the presence of asymmetrical structures in proteins[9–11]; these, however, could be ascribed to heterogeneous alignment of amino acid residues in a protein. A theoretical basis to account quantitatively for this asymmetry of the α-helix remains to be developed.

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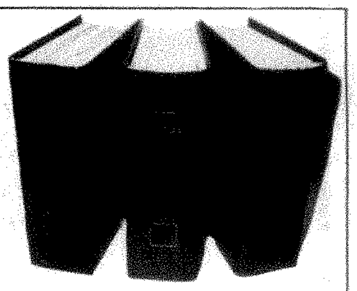

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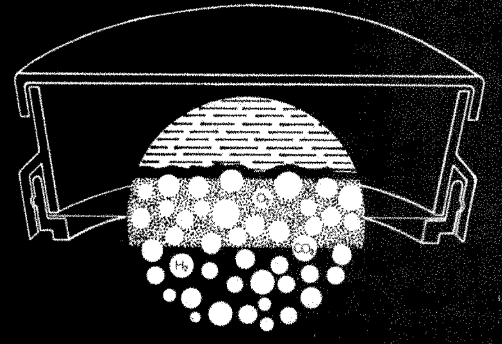

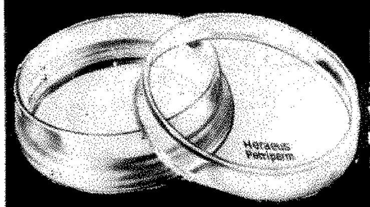

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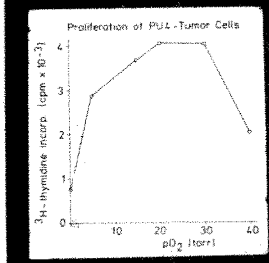

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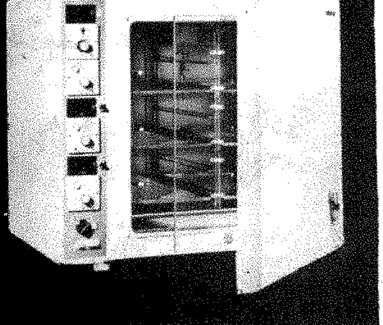

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Palaeogeotherms: implications of disequilibrium in garnet lherzolite xenoliths

FRASER AND LAWLESS[1] have pointed out that, because of the different diffusion mechanisms involved, the two-pyroxene geothermometer[2,3] and the garnet–orthopyroxene geobarometer[4,5] commonly used[6–8] in the interpretation of the pressure–temperature equilibration conditions and depths of derivation of garnet lherzolite xenoliths in kimberlites may be out of phase and thus generate a spurious apparent geotherm. Whilst this may be the case it should, however, be noted that the lines of constant K for garnet–orthopyroxene equilibria in Fig. 1 of Fraser and Lawless[1] are, in the depth range of interest, effectively sub-parallel to geotherms independently calculated[9] from heat flow–production studies. This essentially makes it impossible to determine whether geotherms calculated from xenolith data are real or spurious in the way suggested.

Their Fig. 1 thus highlights the critical importance of the temperature estimates to the depths of origin inferred for such xenoliths. Their further discussion, therefore, purporting to demonstrate the different diffusion blocking temperatures for element exchange reactions between various mineral pairs in garnet lherzolite xenoliths is crucial but quite unconvincing and rather misleading.

Fraser and Lawless attempted to test the possibility that diffusion between pyroxenes and garnets is less rapid than between coexisting pyroxenes by comparing the distribution coefficients for Mg–Fe^{2+} exchange between garnet–clinopyroxene and garnet–orthopyroxene pairs with temperatures based on the Ca–Mg exchange between coexisting pyroxenes[3]. They interpreted the arrays of data points in their Fig. 2 as indicating that Mg–Fe^{2+} exchange between pyroxenes and garnet is effectively blocked below ~1,100 °C whilst lower temperatures continue to be monitored by Ca–Mg exchange between coexisting pyroxenes. In reaching this conclusion they ignored the fact that the xenoliths concerned have probably been derived from an appreciable depth zone in the mantle and hence may be expected to have equilibrated over a range of pressures as well as temperatures. Furthermore, clearly both Mg–Fe^{2+} exchange between pyroxenes and garnets and Ca–Mg exchange between coexisting pyroxenes depend on pressure as well as temperature, although there are uncertainties as to the magnitudes of the pressure dependence of these exchange reactions[2,10–13]. Certainly it is unreasonable for Fraser and Lawless to expect that the xenoliths would plot along lines defining the temperature dependence of the Mg–Fe^{2+} distribution coefficients at effectively one particular pressure. Indeed the experimental evidence of Råheim and Green[12] that $(\mathrm{Mg/Fe^{2+}})_{\mathrm{cpx}}/(\mathrm{Mg/Fe^{2+}})_{\mathrm{gnt}}$ decreases with temperature but increases with pressure suggests that the arrays of points on Fig. 2 of Fraser and Lawless may simply reflect the combined pressure/temperature effects on the distribution coefficients involved.

The variation in $(\mathrm{Mg/Fe^{2+}})_{\mathrm{cpx}}/(\mathrm{Mg/Fe^{2+}})_{\mathrm{gnt}}$ between garnet–clinopyroxene pairs in different metamorphic environments[14,15,19] indicates that Mg–Fe^{2+} exchange is normally effective at temperatures well below 1,100 °C. Krogh[16], Råheim and Green[17], for example, have described both prograde and retrograde variations in this distribution coefficient in eclogite assemblages which indicate that above ~700 °C, diffusion rates even in garnets are fast enough to chemically homogenise the minerals. At lower temperatures Mg–Fe^{2+} exchange is not completely blocked but is restricted to the extent that pronounced zoning is produced and retained, especially in the garnets[16].

Thus contrary to the impression given by Fraser and Lawless and to the interpretation which they place on their Fig. 2, petrographic[16–19] and experimental[2,10–12] evidence indicate that Mg–Fe^{2+} exchange reactions between pyroxenes and garnets probably provide far more sensitive geothermometers below 1,100 °C than the Ca–Mg exchange between coexisting pyroxenes. However, the magnitude of the pressure effect on the Mg–Fe^{2+} exchange reactions in garnet lherzolite assemblages and reliable estimates of the Fe^{2+}/Fe^{3+} ratios in these minerals[19] must first be determined before one can hope to obtain geologically meaningful temperatures from even these geothermometers and hence calculate valid geotherms.

I thank Dr W. L. Griffin for his helpful comments.

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FRASER AND LAWLESS reply—We completely agree[1] with Carswell that the similarity between the temperature dependence of the garnet–orthopyroxene geobarometer[2] and likely geothermal gradients makes it difficult to use this reaction to distinguish between the effects of differences in blocking temperatures and temperature–pressure distributions obtained from samples which have equilibrated at different depths along such geothermal gradients.

In the absence of a suitable alternative geobarometer with a different slope it is difficult to distinguish between these two possibilities or indeed some combination of both. It is, however, unsound to make Carswell's *a priori* assumption that the xenoliths in question are derived from different depths and to use such an assumption to require that the nodules show an appreciable effect of pressure on Fe^{2+}–Mg distribution coefficients. This may indeed be the case as may bulk-compositional effects such as we have mentioned[1]. However, the observed departure of garnet–pyroxene Fe^{2+}–Mg distribution coefficients from the equilibrium values at 30 kbar is equally consistent with increased departure from equilibrium at lower temperature. The extent of low temperature Fe^{2+}–Mg exchange observed in the eclogitic assemblages of Krogh[3] and Råheim and Green[4] results from equilibration over long periods of geological time and cannot be directly compared with the proposed retrograde effects on garnet lherzolite nodules resulting from relatively rapidly changing temperature–pressure conditions during movement towards the Earth's surface before final eruption of the kimberlite.

These important questions can only be resolved by independent tests. The calibration of an alternative geobarometer with different slope is clearly important. Moreover, the bulk chemical homogeneity of the phases in the xenoliths

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should be critically analysed in terms of likely diffusion models and tests should be made of the homogeneity and equilibration of isotopes and refractory trace elements among different mineral phases. Recently published data[5] show a correlation of pyroxene–garnet Mn/Mg and V/Al ratios with clinopyroxene–orthopyroxene temperatures suggesting equilibration of these trace elements. However, the same authors note grain-by-grain relative inhomogeneities of 25% in Cr and Sc contents of the garnets and similar results have been observed elsewhere[6]. Similarly our work has shown that pyroxene–garnet Cr/Al distribution coefficients are widely scattered for the low temperature group of granular nodules but are more tightly grouped in the case of sheared nodules. Isotopic disequilibrium in garnet and spinel lherzolite nodules has also been reported[7–10] although it seems that in the case of garnet lherzolites this applies only to sheared nodules, the granular nodules being apparently equilibrated and giving ages close to that of the host kimberlite[7], despite their dominantly lower temperatures.

There are thus several contradictory pieces of evidence concerning equilibrium in garnet lherzolite and xenoliths and hence uncertainty in their use for the calculation of geothermal gradients. When alternative geobarometers and more isotopic, trace element and kinetic data become available these issues should be more easily resolved.

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Paternal behaviour in fishes: a question of investment, timing or rate?

DAWKINS AND CARLISLE[1] have shown that Trivers' parental investment model[2] of tetrapod maternal care rests on a fallacy, and suggest that maternal behaviour in tetrapods and paternal behaviour in teleost fishes is a result of the relative timing of gamete release. Like Dawkins[3], I view evolution as the selective transfer of information from generation to generation. However, I believe the fundamental difference between the sexes that leads to exclusive maternal or paternal care is the relative rate at which the two sexes can create gametic copies (spermatogenesis requires less time than oögenesis).

In externally fertilising species, optimal sites for zygote development may constitute a limiting resource for spawning individuals, which selects for site constancy and territorial behaviour. Males are favoured as site-constant individuals because in fishes (as in most vertebrates) males are able to produce new batches of fertile gametes and remate at shorter intervals than females[4–8]. A male monopolising a site will leave more descendants than a female occupying that site for the same duration. The male can remate, so roving females still spawn at their maximum rate, and their sons will show the site-constant trait. A territorial male's success is limited by the number of females he can attract; hence the sexual adornment in parental male fishes[9–11]. Parental behaviour evolves as a consequence of acts by the male that promote survival of the zygotes at his site. Where reproduction is not tied to a site, the female will be favoured as the parent because her potential gain from multiple mating is the lesser.

The relative rate of production of gametes is central to the question of sexual selection and follows directly from Dawkins' property of 'fecundity'[3]. Questions of efficiency and energy are not important, except in that they influence the rate of reproduction. The model can be used to explain the evolution of anisogamy, substituting rate for the numerical productivity used by Parker, Baker and Smith[12]. The present model views the evolution of solitary male or female parental care as a cooperative outcome that allows individuals of each sex to maximise their production of offspring.

I thank Jack P. Hailman, Leigh Hurst and Scott Robinson for helpful discussions.

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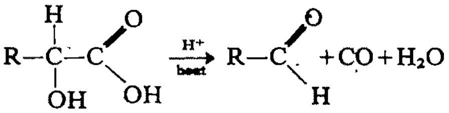
Acid hydrolysis and α-hydroxy acids in meteorites

THE study by Peltzer and Bada[1] of α-hydroxycarboxylic acids in the Murchison meteorite is of considerable interest because if not only presents a different class of molecules to be looked for in meteorite studies, but also provides a possible method for an evaluation of the ammonia content of the environment in which the synthesis of the organic compounds took place. However, I feel that their analyses of the results leave a room to be filled.

They found less α-hydroxy acids in the acid-hydrolysed water extract fraction than in the unhydrolysed fraction. It is known that α-hydroxy acids decompose to aldehyde and CO when boiled in dilute sulphuric acid, with an overall reaction



$$R{-}CH(OH){-}COOH \xrightarrow[\text{heat}]{H^+} R{-}CHO + CO + H_2O$$

As this reaction is known to proceed quite readily, I suspect that this could be the main cause of their observation. Peltzer agreed on this point in his personal communication, saying "the acid-catalysed decomposition of α-hydroxy carboxylic acids probably accounts for the decreased recoveries of the compounds following acid hydrolysis". This in turn would question their conclusion that the hydroxy acids in the meteorite exist primarily in a free or water-hydrolysable state, as their procedure allows whatever α-hydroxy acids are produced through the acid hydrolysis to decompose at the same time to aldehydes. Their conclusion still seems valid, however: Peltzer, in the same personal communication, states that the recoveries of standard compounds at levels equivalent to those found in the meteorite were measured, and that acid hydrolysis recoveries for most hydroxy acids were approximately 10–20% lower than those by water hydrolysis; this extent of the decomposition does not seem large enough to reverse their conclusion.

As the majority of amino acids in meteorites exists in some kind of precursor form and is released only on hydrolysis in 6 M HCl (ref. 2), it would certainly be interesting to study the possible causes for the different states of existence of α-hydroxy acids and of amino acids.

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JEEM

Journal of Embryology and Experimental Morphology

Edited by: R. M. Gaze, London; R. J. Cole, Sussex; R. L. Gardner, Oxford and P. A. Lawrence, Cambridge.

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Theory and Practice in Affinity Techniques

edited by P. V. Sundaram and F. Eckstein

December 1978/January 1979, approx. 250pp., £10.50/$21.75 0.12.677150.0

The book is based on an International Symposium, 'Theory and Practice in Affinity Techniques', held at the Max-Planck Institut für Experimentelle Medizin, Göttingen, in April 1978. The contributors have included information both from their lectures and from the discussions held at the Symposium, while an Editor's Note at the end gives a summary of the discussions on controversial issues in affinity chromatography. The book is a much needed addition to the literature on affinity chromatography and affinity labeling, providing all those concerned with these techniques the chance to learn of the most up-to-date developments from leading workers in the field.

The Society for Applied Bacteriology Technical series No. 11

Techniques for the Study of Mixed Populations

edited by D. W. Lovelock and R. Davies

December 1978/January 1979, xii+228pp., £12.50/$25.75 0.12.45650.2

There has been a considerable increase in interest over the last few years in the interactions between various micro-organisms in particular environments and ecological niches, and also between micro- and macro-organisms. These studies present very different problems from the study of micro-organisms in isolation in pure culture, so that the existing techniques are not entirely appropriate. This book therefore makes a timely contribution to this area, bringing together many methods and techniques which have been developed to study mixed cultures occurring naturally in divers habitats.

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Proceedings of the Serono Foundation Symposia No. 22

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edited by L. Carenza, P. Pancheri and L. Zichella

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Psychoneuroendocrinology is an attempt to provide the essential interdisciplinary approach to research in human reproduction. It involves psychiatrists and psychologists to examine the intrapsychic and psychological determinants of emotions, neuroendocrinologists to relate animal research to clinical studies in man, and obstretricians and gyneocologists to apply these data in the fields of physiology and pathology of reproduction. This book is based on a symposium which brought together people from all these disciplines, to discuss their various approaches to the subject and it clearly demonstrates the need for continued coordination of the different groups involved in psychosomatic research.

Biochemical and Biophysical Perspectives in Marine Biology Volume 4

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reviews

Heavy ion reactions

Ricardo A. Broglia

Nuclear Heavy Ion Reactions. By P E Hodgson. Pp.588. (Oxford University Press, Clarendon. Oxford, 1978) £18

OUR present understanding of the nuclear structure is, to a large extent, based on the information obtained from the analysis of reactions induced by protons, neutrons and light ions, in which the nucleus is moderately excited.

A central feature of the nuclear many-body problem is that the field which confines protons and neutrons is created by the nucleons themselves. This self-confinement is such that the nucleus displays both the degrees of freedom associated with the motion of individual nucleons, as well as collective degrees of freedom where the nucleus reacts as a whole to the external probe. The description of the interplay between the collective and single-particle elementary modes of nuclear excitation constitutes one of the most challenging and exciting problems in the field of many-body physics.

Developments of heavy ion accelerators in recent years have opened the possibility of bringing two heavy ions in contact, thereby making it possible to probe features like the texture of the nuclear surface, and to study nuclear matter under pressures and strains that are only found in the extreme phase of cosmic evolution as, for instance, in the formation of a neutron star.

In his book, Peter E. Hodgson makes a serious atttempt at covering most of the subjects of the new and rapidly developing field of heavy ion reactions Chapter 2 deals with the ion–ion potential Chapters 3, 5, 6, 7 and 8 deal with the phenomena that can arise in gentle collisions, when the two nuclei barely touch each other (elastic and inelastic scattering and particle transfer reactions, that is, grazing collisions) Chapter 9 treats the aspects of gentle collisions associated with intermediate structure. Chapters 4 and 10 are concerned with the phenomena arising when two ions are brought into intimate contact, and where large amounts of energy and angular momentum are transferred from the relative motion to the internal degrees of freedom, leading to fusion processes and to deep inelastic reactions. In the last half of chapter 10 the subject of shock waves and relativistic reactions is briefly reviewed. The book starts with a review of the subject of heavy ion reactions (chapter 1) and concludes with an epilogue which plays both the rôle of a résumé to the book and of an educated guess at future trends and lines of development

The different approaches to all the subjects covered by the book are generously represented, and the referencing, which includes entries up to the first half of 1976, is impressive. These same positive aspects are however also responsible for the fact that this book is a rather confusing catalogue of results more than anything else. In fact, every subject is dealt with by quoting from one or another paper, in many cases not defining even the basic concepts utilised The conclusions of the authors in question are stated without, apparently, worrying whether they are in contradiction, let alone agreement, with something quoted before or after.

For example, in section 2.2 (p97) it is stated, in connection with the contribution of Satchler to the Nashville conference of 1974· "The double-folding procedure has been used with success to analyse the elastic scattering of alpha particles by nuclei, using standard nucleon–nucleon interactions and phenomenological imaginary potentials. When applied to heavy-ion scattering, however, it is found that the doubly folded potential has to be normalised by a factor around 0.5 to fit the experimental data " Ten pages later we read (p108)· "Recent double-folding calculations by Satchler and Love (1976) . . gave potentials agreeing to with 10–20 per cent with those obtained phenomenologically." It is difficult to understand why Hodgson has neglected to tell us that, as a result of the work of Satchler and Love, Christensen and Winther, and Swiatecki *et al.*, we have now achieved a quantitative understanding of the tail of the ion–ion potential, a prerequisite to any further discussion of heavy ion reactions

Another example is provided by the discussion on Coulomb-nuclear interference of chapter 5. In the section which goes under the title of DWBA calculations we read (p272): "The Coulomb-nuclear interference in the inelastic scattering of 72.2 MeV ^{11}B ions by ^{208}Pb . . [is] given quite well by the distorted-wave theory provided the phase of the nuclear excitation form factor is altered by 5° from that given by the collective model." A couple of pages later, in the section on coupled channel calculations, and when quoting the results of Videbæk *et al* on the reaction (^{16}O+^{58}Ni)→(^{16}O(2_1^+)+^{58}Ni) we read that to fit the Coulomb-nuclear interference minimum, multiple step processes have to be invoked. Even so, there remain discrepancies which (p277) "are probably attributable to the neglect of higher-order couplings". Actually, the so-called agreement in the DWBA analysis of the Coulomb-nuclear interference in the reaction (^{11}B+^{208}Pb)→(^{11}B+^{208}Pb(3_1^-)) and the so-called disagreement in the coupled-channel analysis of the reaction (^{16}O+^{58}Ni)→(^{16}O(2_1^+)+^{58}Ni) reflect a rather similar open problem, namely that of the role of multiple step processes in the Coulomb-nuclear interference pattern.

Another weakness of the book is that the priorities given to the different subjects discussed do not, in many cases, reflect their physical relevance. For example, in the middle of section 2.4, in which the calculations of the ion–ion potential are discussed, a method to calculate the imaginary potential is taken up without a word of warning. The subject is given one page (p138) and taken up again under the curious subtitle of: "Other calculations of heavy-ion potentials" (p141); here, another page is spent on it. The fact that the calculation of the imaginary part of the ion–ion potential, assuming it is a well-defined problem, is a formidable task implying the reduction of the full set of coupled equations to a local equation for the probability of the system to remain in its ground state, is not even suggested by the author

One of the most disturbing features of the book is, however, the plain errors it contains—for example, eq. (5.2 3). At first sight it seems we are confronted with the familiar first-order, time-dependent, coupled equations describing the amplitudes associated with the different reaction channels. However, according to the author, these are the equations describing the time evolution of the probabilities associated with each channel. Because of the central role played by the semiclassical approximation in heavy ion physics, this error has serious consequences, both conceptual as well as practical (for example, the incorrect referencing in p265 to standard computer codes)

On p398 the absolute value of the cross section associated with two-nucleon transfer reactions is discussed. The author should have noted that the cross section increases, due to the inclusion of continuum states in the calculation of the corresponding form factors, not by a factor of 25 but rather by a factor of 2. It is correct that in the reference quoted the value of 25 appears. However, in a later publication the same authors acknowledge an error in their computer code, and the new revised value is given. By the way, this value of about 2 is similar to the value found by Feng *et al.*, the reference for which is quoted in p422 of this book, also in connection with the absolute cross section of two-particle transfer reactions It is probably worth noting that an enhancement of the order of 2–4 would be expected from the experience accumulated on this subject in two-particle transfer reactions induced by light projectiles.

According to the cover jacket the book is aimed at graduate students. Let us hope that the excitement which awaits these graduate students entering the field of heavy ion physics, will not be diminished by the rather confusing picture given by this book. □

Ricardo A. Broglia is at the Niels Bohr Institute, University of Copenhagen, Denmark.

Digital image processing

Digital Image Processing and Analysis. Edited by J. C. Simon and A. Rosenfeld. Pp. 513. (Sithoff and Noordhoff: Leiden, The Netherlands, 1977.) $39.50. *Digital Image Processing.* By W K. Pratt. Pp. 750. (Wiley: New York and Chichester, UK, 1978.) £20.80.

IN 1976, a NATO Advanced Study Institute was held at Bonas, in south-western France. *Digital Image Processing and Analysis* contains 38 papers delivered at that meeting, printed from authors' typescripts. Conference proceedings are now commonly produced three or four months after submission of the manuscripts and there really is no excuse for allowing nearly two years to elapse, especially in such a fast-moving field as image processing. There is thus not much in this book that has not already appeared in print elsewhere.

The papers are a mixture of introductory surveys by well known scientists and shorter contributions. Of the former, there is little that is not better set out elsewhere, in textbooks such as Pratt's, for example, or Rosenfeld's own volume (for review, see *Nature* **264**, 142; 1976); of the other papers, a few contain original material—those on recursive filtering in particular—but most of the material duplicates papers published more rapidly and accessibly in journals.

One redeeming feature of the book is the breadth of coverage. Image enhancement and mensuration are covered as well as pattern recognition, and it is certainly healthy to have these intimately related topics in the same volume. Nevertheless budget-conscious librarians can reasonably regard this volume—unlike several other volumes in the NATO ASI series—as dispensable.

Professor Pratt's volume on *Digital Image Processing* is a long and detailed textbook, which has grown out of his image processing course given to electrical engineering students in the University of Southern California. The result of several years' work, it is clear, detailed and authoritative, and—a very valuable feature—the author is aware which topics are difficult to grasp and which can be treated in a reasonably summary fashion. Furthermore, it contains sections on colourimetry and the visual aspects of image appreciation as well as the formal mathematics of image processing. There are sections on image coding and on comparatively recent additions to the field of image processing, such as texture analysis.

The book has the great merit of not only describing the various techniques that are in use but also explaining their purpose and their role in the overall development of this complex subject. As we should expect from a member of the Image Processing Institute, the algebraic aspects of image processing theory are explained clearly and fully but they are not allowed an excessive amount of space.

One criticism can be made, and it is common to most of the recent texts on image processing: the field of electron microscopy, in which the importance of digital image processing has been increasingly recognised during the past few years, is scarcely ever mentioned. This is regrettable, for not only have electron micrographs benefited from some of the techniques described here — originally intended for other types of image—but some quite new procedures have been developed which may well be of interest to students of image processing in general.

This is an extremely well organised and lucid textbook. Despite the author's modest claim that is intended for use as a course text, many workers already familiar with much of the material will find it useful.

P. W. Hawkes

P. W. Hawkes is Maître de Recherches in the CNRS Laboratory of Electron Optics, Toulouse, France.

Magnetospheric physics

Geomagnetic Diagnosis of the Magnetosphere. Physics and Chemistry in Space, Vol. 9. By A. Nishida. Pp.256. (Springer. New York, Heidelberg and Berlin, 1978.)

THE title of this book emphasises the use of geomagnetic data from instruments at ground level for sensing dynamic phenomena occurring far out in the magnetosphere The value of such observations is also stressed in the preface, and the preface explains that the pursuit of this theme has evolved into an "introduction to magnetospheric physics" In both respects the book is successful, demonstrating how magnetospheric physics can be split with only tolerable damage at a timescale of about one second. Although processes typified by anomalous diffusion are important for the slow disturbances, it is not necessary to discuss the mechanisms, which involve higher frequencies

The crudest approximation in plasma physics is hydromagnetics and this provides a useful start Page 1 is indeed a sudden commencement, but coherence persists through to the last pulsating chapter, another hydromagnetic success Much of the intervening chapters is related to reconnection and to particle motion. Dr Nishida has digested a mass of literature on observations and has produced a clear survey, which provides relief after the disjointedness of conference reports Magnetosphere–ionosphere interactions are included and these sections are of particular relevance to observations using the ground-based incoherent scatter technique; this is timely as a large installation, EISCAT, is now being built.

As an introduction to magnetospheric physics this monograph fills a need; it is splendid for new graduate students or others entering the field. It shows the current rapid progress, being made but warnings such as "the problem of the modelling of the dayside reconnection should not be considered to have been settled" are too weak, and more emphasis might have been given to existing controversies. For the substantial research still needed this book will provide a baseline.

J. W. Dungey

J. W Dungey is a Professor of Physics at Imperial College, University of London, UK.

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Dictionary of microbial taxonomy

Dictionary of Microbial Taxonomy. By the late S. T. Cowan. Edited by L. R. Hill. Pp.285 (Cambridge University Press: Cambridge and London, 1978.) £12.50.

TAXONOMISTS, who one might suppose ought to be able to tell us what taxonomy is, seem to agree no more about the meaning than on the derivation of the word. *Taxis* means arrangement: de Caudelle in 1813 coined *taxonomie* and can perhaps by now be forgiven the slip of not spelling it *taxinomie* but he did not explain whether the latter half was from *nomia* (distribution), *nomos* (law) or, mixing Greek with Latin, *nomen* (name). To some, taxonomy means systematics, to others the theoretical study of classification, to others still, nomenclature and the codes which regulate it.

The late S. T. Cowan viewed taxonomy as a scientific art intended to bring order to the often untidy processes of biological classification and nomenclature. Taxonomists he saw as artists—capricious, jealous, argumentative, aggressive—while charitably conceding "Some are good and able to make a useful contribution to science". The microbial taxonomist, he felt, should be a systematist dealing equally with classification, nomenclature and identification—"the trinity that is taxonomy". As a medical and public health bacteriologist, Cowan was often engrossed with practical tasks of identification and he forgave but never forgot the pronouncement of the zoologist, E. Mayr, that identification was the lowest job a systematist did.

Cowan's *Dictionary* contains about 1600 entries, with four introductory chapters, one unfinished, and follows his earlier *Dictionary of Microbial Taxonomic Usage*; the editor, Dr L. R. Hill of the National Collection of Type Cultures, contributes sections on numerical taxonomy. About half of the entries refer to terms or practices in the codes of microbiological nomenclature These international codes, of Nomenclature of Bacteria, Botanical Nomenclature (covering fungi and algae), and Zoological Nomenclature (including protozoa), with the Rules of Nomenclature of Viruses (a provisional virological code was not ratified) differ in numerous confusing ways For example, the type of a bacterial species can be and, very sensibly, usually is a living culture maintained in a reference collection, but the type of a fungal or algal species is generally a dried specimen as required by the Botanical Code. The nomenclatural entries explain the terms, meticulously compare the Codes, where they differ, and give references to the relevant Rules or Articles

Other entries are technical terms of taxonomy, classification and descriptive microbiology (mostly bacteriology), biographies of taxonomists, addresses of culture collections and advice on scientific authorship; just a few (for example, "chemostat") have no discoverable relevance to taxonomy

Many are miniature essays, easy to read and rewarding to the browser, sprinkled with drily entertaining, semi-heretical asides which, for those who did not know Dr Cowan, will convey a flavour of his acrid and sometimes whimsical philosophy. The *Dictionary* is prefaced with an aphorism: "The serious student of nomenclature and the student who takes nomenclature seriously—these are two quite different people", which reads like a 24-carat hand-turned Cowanism but nicely hints that both types of reader should find the book of value—and they will.

J. E. Smith

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Control of eye movement

The Brain and Regulation of Eye Movement. By A. R. Shakhnovich. Pp. 189. (Plenum. New York, 1978) $30

THERE is an important school of Soviet scientists who have studied eye movement. Matyushkin has described the different muscle fibres of the eye muscles. Yarbus has published his studies on eye movement in the examination of patterns, and Luriya used this technique in the investigation of neurological cases. The author of this book, Dr Shakhnovich, has worked on eye movements, especially in the rabbit, on eye movement in man and on the local cerebral blood flow in man This book is of interest chiefly for his account of Russian work, for though he is aware of western work, mostly of the 1950s and 1960s, there are important omissions.

It is not, however, easy to follow the accounts of his own work. Although one can accept that local increased activity can and does increase the blood flow in the cerebral cortex so that there are sharply defined local increases and decreases in flow, there is no successful attempt to relate these changes to the control of eye movement The illustrations, as Lorrin Riggs remarks in his introduction, are frequently devoid of essential detail of scales and experimental conditions. A little more detail on the technique of recording of eye movement by the Yarbus cap would be helpful, as Yarbus's own account, though admirable, is not readily available here

In many current problems the author has little help to provide. The function of slow contracture fibres in the eye muscles remains obscure, and the question of vision during saccades is not clarified As Riggs also remarks, the elegance of the bioengineering approach to the control of eye movement is not exploited in this book.

It is, however, his account of the literature which is unhelpful. "Stimulation" of unknown technique in poorly defined places does not provide a useful basis for study of the controlling mechanisms of eye movement in the midbrain, and it is difficult to follow his argument on the vexed question of reflex responses to stretch in eye muscles.

D. Whitteridge

D. Whitteridge is Professor of Physiology at the University of Oxford, UK.

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Double stars

Double Stars. By W. D. Heintz. Pp. 174. (D. Reidel: Dordrecht, The Netherlands, 1978.) Hardback Dfl.65; $29; paperback Dfl.30; $14.

DOUBLE STARS in the sky represent objects of great interest to the astronomer—not only because of their prodigious abundance (more than three-quarters of all stars around us seem to be multiple), but also because a symbiosis of their components provides the best data with which to document our theories on stellar evolution. Observations (positional photometric, spectroscopic) of binary systems have provided almost all we know on the masses and absolute dimensions of the stars, and, in addition, have enabled us to gain more subtle empirical insights into their structure.

In spite of these facts, the students of this important branch of astronomy have so far been served none too well by the existing contemporary literature. For this reason alone the appearance of the book under review is to be welcomed, because in many aspects it usefully fills a noticeable gap. However, this welcome cannot be extended to it without reservation concerning certain parts of its contents, where the latter falls short of the actual needs.

The present reviewer has nothing but praise for the first half of the book concerned with wide (visual or astrometric) binaries—a field in which its author is a recognised expert. That part of its text presents the first comprehensive picture of the field since R. G. Aitken's *Binary Stars* of 1935 (Dover reprint, 1964), brought up-to-date by an account of more recent developments (such as the use of electronic cameras, speckle interferometry, and so on). All this should be very useful to the student and researcher alike—not only by what it describes, but also by many references to the original sources which can guide its user in further reading.

With his account of the close (that is, spectroscopic and photometric) binaries, contained in the second half of the book, the author has been somewhat less fortunate—perhaps because its contents constitute largely a second-hand report which is in many places quite out of date. Thus, on the methodical side (determination of the elements) it does not go really beyond what one could find in Aitken's 1935 classic: Had there been no developments of the subject since that time, would it have been worthwhile to write another book about it? Of course they were; and some of them spectacular; but the author went past them without much notice.

Thus, the reader of Heintz's book will not learn much from it alone on the effects of distortion (or reflection) on the determination of masses or absolute dimensions of stars constituting close pairs. He will not learn that the process of "rectification" of their light curves represents an empirical make-shift, without theoretical justification, which is apt seriously to falsify the original light message. Hydrodynamical phenomena (gas streams) in close binary systems are still treated in terms of particle mechanics (in which the pressure is ignored); and photometric effects are ascribed to them without appropriate inquiry as to the density of gas which could possess the requisite optical depth.

Moreover, in the last part of the book, the reader is being exposed to various evolutionary 'scenarios' without sufficient caution about their uncertainty or hypothetical nature. This part of the book is certainly not a safe guide for the uninitiated. However, if it succeeds in making him at least interested enough in this field to follow it up by further reading (or work) of his own, it will have satisfied a very useful aim.

Zdeněk Kopal

Zdeněk Kopal is Professor of Astronomy in the University of Manchester, UK.

Basic introduction to pesticides

Pesticides: Preparation and Mode of Action. By R. Cremlyn, Pp. 240. (Wiley: Chichester, UK, and New York, 1978.) £12

THIS book is aimed specifically at students who are taking first degrees or other qualifications in agriculture or related subjects. It covers, in separate chapters, insecticides, fungicides, herbicides, fumigants, rodenticides, nematicides, molluscicides; and the newer methods of insect control. The basic pattern of these chapters is to start with a brief history of how each particular group of pesticides came to be developed, to give brief details of synthetic routes, and to consider the biological activity.

As with so many agricultural subjects, a wide range of the traditional academic disciplines is involved, from organic chemistry to ecology. The book is obviously written by a chemist: I felt somewhat uneasy with some of the more biological statements. For example, "The nervous system is characteristic of mammals and insects . . . " True, but incomplete, and the uninitiated might wonder which other groups of animals have nervous systems. I am not a professional chemist, but the accounts of synthetic routes seemed to be brief and competent. However, I was less happy with some other aspects of the chemistry. Thus, on p56 DDA is declared to be a metabolite of DDE—as far as I am aware DDA is metabolised only from DDT. Aldrin and dieldrin are both declared (on p64) to be chemically very stable: obviously it depends on how one defines stability, but dieldrin is normally regarded as more stable than aldrin.

These chapters are illustrated with many structural formulae, which are useful, but identification is inconsistent. Some formulae are named, but many which can be identified by short names bear only a number. This number has to be discovered in the text before the identity of an unknown compound is revealed, which is tedious. There are also 28 black-and-white photographs, all taken by well-known manufacturers of pesticides, and mostly of crops with and without pesticide treatment They are of somewhat marginal value.

Two introductory chapters discuss broader issues that apply to the whole range of pesticides. One, on physico-chemical factors, discusses briefly the important topics of pesticide design, formulation and selectivity, application and behaviour of pesticides in the environment. The other covers biochemical reactions that are relevant to the mode of action of pesticides: respiration, photosynthesis, conduction of nerve impulses, transport in plants, synthesis of proteins.

The penultimate chapter considers pesticides in the environment, and crystallised some of the reactions I had experienced from the earlier chapters. Facts are often presented in a slightly misleading way, and often key references are absent. References are indeed rather few in number. They are placed at the end of each chapter, and the same standard texts such as Corbett, Hartley and West, Martin and O'Brien recur frequently. This book is of course more up-to-date than its predecessors, but does not replace them.

In summary, I cannot regard this as an authoritative book, but it may be useful for students who do not want to delve too deeply into any aspect of the subject.

F. Moriarty

F. Moriarty is a Principal Scientific Officer at the Institute of Terrestrial Ecology, Monks Wood Experimental Station, Huntingdon, UK.

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by **A.R. Ubbelohde, C.B.E., F.R.S.**, *Department of Chemical Engineering and Chemical Technology, Imperial College, London*.

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what's new—radioimmunoassay and biochemicals

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A feature on the measurement of radioactivity will appear in the 18 January 1979 issue.

Bethesda Research Laboratories. The BRL sequencing gel electrophoresis system for 34×40 cm gels, comes complete with comb and spacers, glass plates, 12 clamps, platinum electrodes and electrical leads. The support unit measures 17 inches high × 14 inches wide × 15.5 inches deep, overall. The vertical gel support measures 35×43 cm, and features a 22×25 cm cut out facilitating uniform heat dissipation from the sandwich during electrophoresis. The buffer chambers hold approximately 500 ml each. After polymerisation, the gel sandwich slides easily into place forming a liquid-tight seal with the upper buffer chamber by the unique silicone rubber gasket system. The gel dimensions are compatible with standard medical X-ray film, a convenience when preparing autoradiographs.
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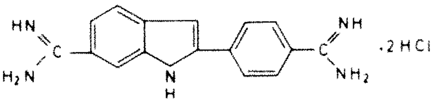

Thus, DAPI will bind differentially to yeast mitochondrial and nuclear DNA forming highly fluorescent complexes and enhancing the separation of such DNAs in caesium chloride gradients. In tissue culture cells DAPI is rapidly taken up into cellular DNA, yielding highly fluorescent nuclei and no detectable cytoplasmic fluorescence. If, however, the cells were contaminated with mycoplasmas, discrete fluorescent foci were readily detected in the cytoplasm and on the surfaces of the cells. DAPI staining of cells infected with vaccinia virus reveals characteristic fluorescent virus 'factories' in the cytoplasm. In a similar way large DNA-virus, phages and bacteria can be stained and in yeast cells petite mutants without mitochondria can be clearly distinguished. Furthermore, trypanosomes and mesenchymal mammalian tissues can be stained. DAPI is soluble in water at $\sim 2.5\%$. Other new products from AC & S include four additions to the range of Dako-Accurate antibodies. (1) Monospecific rabbit anti-human placental lactogen chorionic somatomammotropin. (2) Antibodies to whole sera, rabbit anti-fetal calf serum protein. (3) Antibodies to animal antigens, rabbit anti-horse ferritin (spleen). And (4), horseradish peroxidase conjugated, rabbit anti-guinea pig immunoglobulins.
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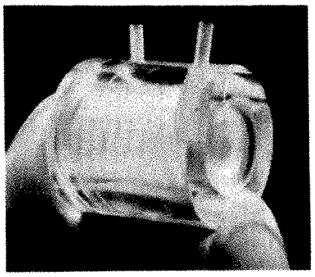
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The person appointed should take up duties on October 1, 1979

Further information may be obtained from the Registrar (Room 214, Whiteknights House), The University, Whiteknights, Reading RG6 2AH, by whom applications should be received not later than January 31, 1979. 8847(A)

UNIVERSITY OF LONDON
Imperial College of Science and Technology

DEPARTMENT OF MATHEMATICS

LECTURESHIP IN MATHEMATICS

The department has a vacancy for a Lecturer in mathematics effective from October 1, 1979 or some mutually agreeable date. The current salary scale is £3,909 to £7,754 plus London allowance £450 per annum

The teaching duties of the Lecturer will include lecturing to students of engineering, including those in the new four-year course, and to students of mathematics

Candidates should have research interests in one or more of the applications of mathematics, in which the numerical and computational aspects of the subject play an essential role. The present interests of the applied areas of the department include theoretical fluid mechanics, continuum mechanics, theory of condensed matter and statistics. Preference will be given to applicants with an interest in one or more of these areas and to younger applicants

Applications (no form) should be sent by February 5, 1979 to Professor J T. Stuart, F.R.S, Department of Mathematics, Imperial College, Queen's Gate, London SW7 2BZ, from whom further particulars can be obtained. 8852(A)

CHROMATIN RESEARCH
BIOPHYSICS LABORATORIES
Portsmouth Polytechnic

RESEARCH ASSOCIATE

required for two years initially, to work in Dr E M. Bradbury's research group. The structure and function of chromatin are studied by both physical and biochemical methods. The group has a high field NMR spectrometer and is actively engaged in neutron and X-ray diffraction work. Experience of one of these techniques, although desirable, is not a prerequisite for appointment However, knowledge of, and some research experience in general biochemical techniques is expected. The successful candidate's project will depend on her/his interests and background.

Salary scale· £3,510 to £3,609 per annum.

Application forms and further details may be obtained from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth PO1 2QQ, to whom completed applications should be returned by December 30, 1978 quoting reference S55.

8795(A)

UNIVERSITY OF SIERRA LEONE
Fourah Bay College

Applications are invited for

TWO LECTURERS

for the

DEPARTMENT OF GEOLOGY

Applicants bould have a good honours degree in Geology and postgraduate qualifications preferably at Ph D. level The preferred fields are as follows I) Palaeontology, but candidates should also have experience or interest in Sedimentology and Stratigraphy; II) Tectonics and Structural Geology; but candidates should also have interest or experience in Archaean Geology. In addition to teaching duties, appointees will be expected to develop postgraduate and mapping courses in their subjects and organise and lead field excursions.

Salary Scale. Lecturer, Le3,740 to Le6,270 p.a. (£1 sterling=Le2.10) The British Government may supplement salaries in range £6,054 to £7,170 p a (sterling) for married appointees or £4,020 to £4,926 p a. (sterling) for single appointees (subject to the Government of Sierra Leone's approval—reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Superannuation or contract terms, outfit allowance Le120 (where applicable), car allowance Le600 p a.; annual leave; family passages; car loan negotiated. Part-furnished accommodation at reasonable rental

Detailed application (2 copies) with curriculum vitae naming three referees to be sent direct to Secretary, University of Sierra Leone, Freetown, Sierra Leone, by January 14, 1979.

Applicants resident in the UK should also send a copy to the Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT Further particulars may be obtained by writing to either address.

8856(A)

AGRICULTURAL RESEARCH COUNCIL
PLANT BREEDING AND GENETICS

A vacancy will arise shortly for a

Scientific Adviser

to the Secretary of the A.R.C. Applications are invited from candidates who would like to join the small team of senior scientists which advises him on the scientific aspects of research administration.

The post is graded Senior Principal Scientific Officer with a salary scale of £10,043 to £11,300 plus £465 for working in Central London. Superannuation is under a non-contributory scheme analogous to that of the Civil Service.

The duties of the Adviser will lie in the field of plant breeding of agricultural and horticultural crops including fodder crops and grasses, and the relationship of breeding programmes to the agronomy of field crops and forages. A proved record of research is required, preferably in plant breeding, but candidates whose experience lies in agronomic aspects of the field will also be considered.

Applications from candidates with a good honours degree or equivalent qualifications and a successful record in plant breeding and genetics, should be by letter, accompanied by a full curriculum vitae and the names of two referees.

ARC

Further particulars from Miss M. J. Gard, Agricultural Research Council, 160 Great Portland Street, London W1N 6DT. Closing date January 26, 1979. 8814(A)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND
DEPARTMENT OF BIOLOGY

FISHERIES BIOLOGY

Applications are invited for a position open at senior rank, in the area of Fisheries Biology Candidates must demonstrate a strong record of research and publication in fisheries, and a successful background in supervision of fisheries research at the M Sc. and Ph.D. levels. The successful candidate will play a key role in development of a new fisheries program.

ASSISTANT PROFESSOR—AQUATIC ZOOLOGY

Applications are invited for a position open at the rank of Assistant Professor, in the area of Aquatic Zoology. Enquiries are especially welcome from candidates with specialisation in any of the following areas: marine and freshwater fishes, limnology; planktology; fish population dynamics.

Applicants should submit a curriculum vitae together with the names of three referees. Applications close on January 31, 1979 with Dr G. R. South, Head, Department of Biology, Memorial University of Newfoundland, St John's, Newfoundland A1B 3X9, Canada. 8787(A)

UNIVERSITY OF HONG KONG

Applications are invited for the following new posts which, it is hoped, will be established from September 1, 1979.-

LECTURESHIP/ASSISTANT LECTURESHIP IN PHYSICS

Applicants should have a Ph D degree in physics and be carrying out active research in any field of physics.

STAFF TUTORSHIP IN PHYSIOLOGY

The candidate selected for appointment will be expected mainly to assist in the teaching of Medical Laboratory Science.

Annual salaries (superannuable) are:-

Lecturer; Staff Tutor: HK$63,540 by 4,260 to 72,060 Bar 76,320 by 4,260 to 106,140; Assistant Lecturer: HK$46,260 by 4,320 to 59,220. (£=HK$9.30 approx)

Starting salary will depend on qualifications and experience.

Further particulars and application forms may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or the Assistant Secretary (Recruitment), University of Hong Kong, Hong Kong.

Closing date for applications for all posts is January 31, 1979. 8815(A)

Technicians

Animal Health Laboratories—Production

Two Technicians are required in our recently established production laboratories at Milton Keynes to fill important and challenging posts in the bacterial vaccines production department. The work, which is concerned predominantly with the preparation and purification of bacterial antigens on a large scale, is suitable for persons with either HND, HNC or pass degree qualifications in appropriate scientific subjects and, although prior experience in a microbiological or production field would be an advantage it is not essential, as full training will be given.

Pharmaceutical Research—Drug Analysis

A Technician is required in a research department involved in the development of analyses for new drugs. The department is well equipped with gas chromatography, high pressure liquid chromatography and mass-spectrometry. Experience in any or all of these techniques would be an advantage but training will be given to a suitable applicant.

We are part of a large expanding international company in which salaries and job security are of the high standards to be expected in such an organisation. Other benefits in our employment package include minimum of four weeks holiday, contributory pension scheme with free life insurance, free membership of PPP, flexible working hours, staff restaurant with meal vouchers, sports and social club facilities and contribution to removal expenses where appropriate.

Please write or telephone for an application form to: Adrian Forrest, Hoechst UK Limited, Walton Manor, Walton, Milton Keynes, Bucks. Tel: Pineham 5068.

8827(A)

Hoechst

POSTDOCTORAL POSITION

Ph D. in Biochemistry or Chemistry for studies on the mechanism of enzyme action by direct inspection of reaction intermediates Experience in rapid kinetics techniques, organic synthesis (peptide and nucleotides) and enzymology (particularly metallo-enzymes) desirable

Send resume and two letters of recommendation to David S Auld, Department of Biological Chemistry, Harvard Medical School, Seeley G Mudd Building, 250 Longwood Avenue, Boston, Massachusetts 02115

Affirmative Action/Equal Opportunity Employer 8863(A)

POSTDOCTORAL POSITION

available immediately for biochemical and genetic studies of viral interference with influenza Ph D and research experience in virology and/or biochemistry preferred

Submit curriculum vitae to Dr Irene T Schulze, c/o Personnel Services, St Louis University Medical Center, 3556 Caroline Street, St Louis, MO 63104

EEO/Handicap Employer-M/F-AA
8780(A)

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

(University of Reading)

NUTRITION DEPARTMENT

A graduate is required to participate in a programme of fundamental research into the immunology of the gastrointestinal tract The work will be mainly concerned with hypersensitivity reactions to food antigens, their development and regulation The appointed person will work within a small immunology unit and will be expected to initiate and execute experimental work with a minimum of supervision.

Qualifications: First or Upper Second class Honours Degree in biological sciences with emphasis on cell biology or biochemistry Applicants with some experience of immunological techniques will be at an advantage

Appointment will be as Scientific Officer (£2,839 to £4,415) or Higher Scientific Officer (£4,101 to £5,445) according to qualifications and experience At least two years' relevant post-qualifying experience is required for appointment as H S O Non-contributory superannuation scheme

Application forms are obtainable from the Secretary, N I R D, Shinfield, Reading RG2 9AT. Quote reference 78/40. 8835(A)

UNIVERSITY OF CALIFORNIA, LOS ANGELES FACULTY POSITION

in the

DEPARTMENT OF ASTRONOMY UCLA

The Department of Astronomy invites applications for a regular faculty position beginning Fall, 1979. The initial level of this position is open and dependent on the qualifications of the applicant.

Applicants with an instrumental/observational background (including space astronomy) are preferred, but applicants from all fields of astronomy will be considered.

A list of publications, copies of preprints, 3 letters of recommendation, and a statement of research interests should be submitted in support of the application.

Deadline for application is March 1, 1979 UCLA is an Equal Opportunity/Affirmative Action employer

Mail applications to
Dr Roger K. Ulrich,
Chairman,
Department of Astronomy,
University of California,
Los Angeles,
405 Hilgard Avenue,
Los Angeles, California 90024
8864(A)

PUBLIC HEALTH LABORATORY SERVICE BOARD

61 Colindale Avenue,
London NW9 5EQ

DIRECTOR GENETIC MANIPULATION LABORATORY

P H L S CENTRE FOR APPLIED MICROBIOLOGY AND RESEARCH, PORTON DOWN

The P H L S Board invite applications for the post of Director of the Genetic Manipulation Laboratory at the civil microbiological laboratories to be established at Porton from April 1, 1979.

The appointment will be in the grade of Principal Microbiologist The successful candidate should be a postdoctoral molecular biologist with experience in genetic manipulation techniques, experience in the use of containment facilities at the Category 3 or 4 level is essential He/she will be responsible for research into improved methods of carrying out genetic manipulation in microbes and for supervision of the secure facilities at the Centre available for experiments in genetic engineering

Salary scale £6,999 to £9,177 N.H S. term and conditions of service

Applications with the names of three referees should be sent to Dr R A Bassett by December 29, at the above address, from whom further details may be obtained.
8851(A)

UNIVERSITY OF ST ANDREWS

DEPARTMENT OF COMPUTATIONAL SCIENCE

Applications are invited for a lectureship in the Department of Computational Science, tenable from September/October, 1979. Candidates should have a Ph.D or equivalent experience in Computer Science Research.

Salary at appropriate point on scale £3,883 to £7,754, starting salary probably not above £5,604, U S S /F S S U.

Applications (two copies preferably in typescript) with the names of three referees should be lodged by February 2, 1979 with the Establishments Officer, The University, College Gate, St Andrews, Fife, from whom further particulars may be obtained.
8793(A)

AN DER FREIEN UNIVERSITÄT BERLIN

IST FOLGENDE STELLE ZU BESETZEN

FACHBEREICH 21 CHEMIE, INSTITUT FÜR KRISTALLOGRAPHIE

ORDENTLICHER PROFESSOR (AH 6)

Als Forschungsgebiete kommen in Frage Aufklarung der Konstitution von Polymeren (Biopolymere bzw synthetische Polymere, moglichst in Zusammenarbeit mit den Forschungsschwerpunkten in der Biochemie bzw. Makromolekularen Chemie) oder Kristallographie der Oberflachen (moglichst in Zusammenarbeit mit den Forschungsschwerpunkten in der Mineralogie Analytischen Chemie oder Physikalischen Chemie). *Einstellungsvoraussetzung* § 13a HSchLG Berlin Bewerber sollen bereit sein, sich kollegial an den Lehr- und Forschungsaufgaben in der Kristallographie sowie an den Selbstverwaltungsaufgaben des Fachbereichs zu beteiligen Bewerbungen mit den ublichen Unterlagen sind innerhalb von 6 Wochen zu richten an den Vorsitzenden des Fachbereichs Chemie, Takustrabe 3, 1000 Berlin 33. 8804(A)

UNIVERSITY OF ZAMBIA

Applications are invited for the post of:

CHIEF/SENIORTECHNICIAN
IN THE DEPARTMENT OF PATHOLOGY AND MICROBIOLOGY—SCHOOL OF MEDICINE

Candidates should have an A I M L T. or F I M.L.T. or equivalent. Duties will be (a) to assist in the organisation/preparation of practical classes for Medical and Para-Medical students (b) to teach Para-medical students (c) to participate in the routine diagnostic services at the University Teaching Hospital

Salary Scales: Senior Technician. K4,416 to 5,136 p a ; Chief Technician: K5,316 to 6,324 p.a. (£1 sterling= K1 55) The British Government may supplement salary in range £3,828 to £4,182 p a. (sterling) for married appointee or £1,836 to £1,986 p a (sterling) for single appointee (reviewed annually and normally free of all tax) and provide childrens education allowances and holiday visit passages. Family passages, superannuation scheme, biennial overseas leave, housing allowance, various allowances

Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Registrar, University of Zambia, P.O Box 2379, Lusaka, Zambia by January 13, 1979 Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

8813(A)

UNIVERSITY OF LEEDS, Department of Metallurgy, TECHNICIAN GRADE 5 Applications are invited for a Technician Grade 5 to assist in the operation and maintenance of Electron Microscopes within the Department of Metallurgy. The Department makes extensive use of electron microscopes in both teaching and research, and, after appropriate training, the person appointed would be expected to assist in specimen preparation and instrument operation as well as performing routine servicing of microscopes and their ancillary equipment. Previous experience in electron microscopy is not required but applicants should have knowledge in one or more of the following areas operation or maintenance of electron microscopes or similar equipment; electronics; photography, vacuum systems. Applicants should have H N.C or equivalent qualifications and have had substantial relevant experience Salary on the scale £3,186 to £3,720 a year (under review). Applications, with full details of previous experience, should be addressed to Professor J. Nutting, Department of Metallurgy, The University, Leeds LS2 9JT, West Yorkshire

8826(A)

ST MARY'S HOSPITAL MEDICAL SCHOOL
(University of London)

MEDICAL LABORATORY SCIENTIFIC OFFICER
(Technician)

required in Department of Chemical Pathology to assist with research project of 3 years duration, on oestrogen metabolis in post-menopausal women Work will involve some isotope studies and some radioimmunoassay. Commencing salary within range £3,261 to £3,813 plus £354 per annum London Allowance Apply, The Secretary, at above address quoting Ref. MLSO/CP/N.

8831(A)

TRINITY COLLEGE DUBLIN
GENETICS DEPARTMENT

RESEARCH FELLOW

A postdoctoral fellow is required for studies on the control of cell division in yeast, supported by a training grant from the Medical Research Council of Ireland Salary is £3,300 per annum tax free.

Applications with curriculum vitae and the names of two referees to Dr B L. A Carter, Genetics Department, Trinity College, Dublin 2, Ireland.

8805(A)

STATE OF KUWAIT
KUWAIT UNIVERSITY

Applications are invited for academic posts at Kuwait University. Contracts for two years renewable for a further period of four years if convenient to both applicant and the University.

Applicant should be.—

- a) Holder of Ph D. degree or its equivalent in the respective specialisation.
- b) Holder of an academic post at present in an accredited university or research centre

It should be noted that:—

- — Teaching in English is *a must.*
- — Method of teaching at Kuwait University is based on the credit system.

Field of specialisation —

Botany and Microbiology Department· Virology

Geology Department· Environmental Geology/Geomorphology.

Qualifications include experience in teaching environmental Geology and Geomorphology and willingness to participate (on rotating basis) in instructing elementary courses in marine, geostatistics and introductory geology The candidate will be expected to assist in developing research programmes in Geology and teaching in the Graduate School which opens shortly.

Rank and salary will be determined according to present position, qualification and years of experience.

Monthly total salaries are in the range of.—

Lecturer (U.S. Asst. Professor): K.D 460 to K D. 550

Asst. Professor (U.S. Assoc Professor): K.D 575 to K.D. 665.

Professor: K.D 665 to K.D. 755.

(One K D. is equivalent to U.S. Dollars 3.5. There is no income tax in Kuwait, currency is freely transferable without any restriction).

Candidates are also entitled to the following privileges —

1. Annual return air tickets to the country of citizenship would be provided to the applicant, his wife and three of his children not exceeding the age of twenty
2. Free furnished accommodation with water and electric supplies.
3. Excess baggage allowance in the vicinity of 30 (thirty only) kilogrammes for the staff member and 20 (twenty only) kilogrammes for each member of his family shall be incurred by the University, provided these members accompany him upon his joining the service.
4. The staff member shall be entitled to free sea freight allowance of 400 (four hundred only) kilogrammes, provided costs of the said freight for the said cargo do not exceed Kuwaiti Dinars 100 only. This is applied at the beginning and end of the contract.
5. Gratuity at the end of service shall be one month's basic salary for each year spent in the service of the University.

Application and Curriculum Vitae forms are obtainable from the nearest Kuwait Embassy Completed application accompanied by three letters of recommendation, together with a non-returned copy of the candidate's publications should be submitted *directly* to Kuwait University, Department of Administration Affairs, P O Box 5969, Kuwait Short terms contract (e.g. sabbatical, visiting, etc., will also be considered.

8838(A)

UNIVERSITY COLLEGE CARDIFF
DEPARTMENT OF PHYSIOLOGY

LECTURER IN PHYSIOLOGY

Applications are invited from those qualified in medicine, dentistry, physiology, or related disciplines for a Lectureship to begin on April 1, 1979 or as soon as possible thereafter The salary will be within the non-clinical lecturer range, with membership of U.S.S , at a starting point determined by the applicant's age and experience: £3,883 to £7,754 (medically qualified applicants normally at not less than 3rd point on scale).

Applications (2 copies), together with the names and addresses of two referees, should be forwarded to the Vice-Principal (Administration) and Registrar, University College, P O Box 78, Cardiff CF1 1XL, from whom further particulars may be obtained Closing date January 16, 1979. Please quote reference 1659. 8816(A)

UNIVERSITY OF MELBOURNE
LECTURESHIP
in the
DEPARTMENT OF OPTOMETRY

Applications are invited for the above-mentioned position in the Department of Optometry of the University of Melbourne.

Applicants should have a higher degree and a demonstrated capacity for research. The field of interest in which the appointment will be made is open. Applications are therefore sought from optometrists with interests in either the clinical or pre-clinical sciences, from physicists with post-graduate training and experience in optics, and from physiologists with developed interests in the neurobiology of vision.

SALARY: $A15,179 to $A19,940 per annum.

Enquiries about the Department may be directed to the Chairman, Professor B L Cole, in the University

Further information, including details of application procedure and conditions of appointment, is available from the Registrar, University of Melbourne, Parkville, Victoria, 3052, Australia, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications close on February 28, 1979. 8785(A)

CITY OF BIRMINGHAM POLYTECHNIC
Faculty of Engineering and Science
DEPARTMENT OF SCIENCE

PRINCIPAL LECTURER
in
BIOMEDICAL SCIENCES

required to contribute to the teaching of courses in Medical Laboratory Sciences and Biological Sciences and to make a significant contribution to the development of Medical Laboratory Science Courses in the Science Department.

Candidates should have suitable academic qualifications together with experience of teaching at degree level, and will be expected to contribute to research programmes within the department.

An ability to contribute to the teaching in one or more of the following areas of study would be an advantage: Virology, Immunobiology, Genetics, Cell Biology, Serology.

Salary Scale: Principal Lecturer: £7,047 to £7,818 (bar) to £8,844.

Further details and application forms (to be returned by January 5, 1979) from: The Personnel Officer, City of Birmingham Polytechnic, F Block, Perry Barr, Birmingham B42 2SU. (021-356 6911 Ext. 217). 8786(A)

Colaiste na hOllscoile Corcaigh
UNIVERSITY COLLEGE CORK

The Governing Body invites applications for the following offices: —

PROFESSORSHIP OF BOTANY
PROFESSORSHIP OF NUTRITION

Salary range (from March 1, 1979) is: £9,775 to £11,305 per annum.

Application forms and further details of the posts may be obtained from the undersigned.

Latest date for receipt of applications is *Friday, January 19, 1978.*

M. F. Kelleher
Secretary

8806(A)

THE PAPUA NEW GUINEA UNIVERSITY OF TECHNOLOGY

CHAIR OF FORESTRY

Applications are invited for the Chair of Forestry and Head of Department of Forestry in the University of Technology, Lae, which will fall vacant at the end of 1978.

DUTIES AND RESPONSIBILITIES:— The successful candidiate will be responsible for continuing the development of the Forestry degree course, for the formulation and carrying out of an applied research program in the Department, and for maintaining the closest links with the national forest service and with the forest-based industry. The basic degree course, started in 1975, is now well-established but may need to be evaluated and modified to meet the relevant and changing requirements of a developing Papua New Guinea.

Applicants must have a postgraduate qualification and considerable experience and expertise in tropical forestry, particularly in integrated land-use in the tropics, and must have appropriate teaching, research and administrative experience in a developing country. Academic calibre should be demonstrated by research and publications.

SALARY AND CONDITIONS OF SERVICE:— The salary will be that of a Professor K20,460 per annum. Allowances additional to salary are payable as follows:— Married K2,300 per annum; single K1,300 per annum. In certain circumstances a child allowance of K156 per annum is also payable. An additional allowance and additional fares may be provided for children being educated away from their parents' place of residence. Other benefits include furnished housing (hard goods only) supplied at nominal rental, leave fares to place of recruitment every second year and equivalent fares to Canberra, Australia, each alternate year and six weeks annual leave. Superannuation benefits apply in most cirmustances. Appoinment will be on a contract basis for a maximum of six years. Terms and conditions of service are currently under review.

APPLICATIONS:— Applications should comprise a full curriculum vitae including particulars of age, nationality, marital status, dependents, qualifications, experience, publications and also the names and addresses of three referees from whom confidential enquiries can be made.

Photo copies of certifying documents should be submitted with the applications addressed to the Registrar to reach this office by February 2, 1979.

Those applying from the United Kingdom should also submit a copy of their application to the office of the Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

Further information is available from the offices of the A.C.U. or from the Registrar of the University.

The University reserves the right to make no appointment or to make an appointment by invitation at any stage.

The Registrar
The Papua New Guinea University of Technology
P.O. Box 793
LAE, PAPUA NEW GUINEA

8858(A)

UNIVERSITY OF SOUTHAMPTON

Computer Studies Group

Research into Relational Databases

Over the last 5 years a relational database management system (RDBMS) has been developed in prototype form by a joint team from ICL, the BBC and Southampton University. A three year research project to investigate and improve the efficiency of the RDBMS system has been financed by the Science Research Council, and applications are invited for the posts of RESEARCH FELLOW and RESEARCH ASSISTANT to work under the direction of Professor D W Barron. Candidates should preferably have a first degree in Computer Science and must have experience of programming in a language such as Pascal, Coral 66, Algal W or Algol 68. For the Fellowship a Ph D in a relevant branch of Computer Science, or extensive relevant experience, is required.

Salary: Research Fellowship from £3,883 (Scale 1A)
Research Assistantship from £3,384 (Scale 1B)
according to qualifications and experience.

Further particulars are available from Miss E C P Roberts, Staffing Section, The University, Southampton SO9 5NH.
Please quote ref. MAI/2/Na.

8788(A)

COMMONWEALTH MYCOLOGICAL INSTITUTE

FERRY LANE, KEW, SURREY

Vacancies for

FOREST MYCOLOGIST and PLANT PATHOLOGIST

THE FOREST MYCOLOGIST will work on the fungal pathogens of Central American pines, in a post funded by the UK Ministry of Overseas Development, for a period of three years.

The successful candidate will be based at the Commonwealth Mycological Institute, but will be required to make annual collecting visits to Central America to survey and collect fungi associated with pines.

Qualifications: A good honours degree with postgraduate experience involving work on the identification of fungi. Experience in forest pathology will be an advantage.

THE PLANT PATHOLOGIST will work as a Liaison Officer under the UK Ministry of Overseas Development, based at the Commonwealth Mycological Institute.

He will assist developing countries on behalf of ODM with the identification of pathogens of forest trees; advise on control methods and supply information of current interest on forest pathology; conduct short-term surveys and make advisory visits overseas. He would be expected to contribute to the work of the Commonwealth Mycological Institute.

The post forms part of a small team comprising two Liaison Officers and two ancillary staff.

Qualifications: Preference will be given to a forest pathologist with knowledge of plantation problems. Tropical experience is essential.

Salary, FOR BOTH POSTS, in the scale £2,839 to £8,461 (which is subject to revision). Starting salary according to age, qualifications and experience.

Application forms, and further particulars, obtainable from: The Executive Director, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN.

Closing date for applications: February 12, 1979. 8844(A)

UNIVERSITY OF LONDON

CHAIR OF MATERIALS AT IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

The Senate invites applications for the above Chair, tenable in the Department of Metallurgy and Materials Science.

The Professor of Materials will take a leading part in teaching, curriculum development at the undergraduate and postgraduate levels and in initiating research. It will be an important function of the Chair to foster cooperation in the subject with other Departments of the College and to maintain links with industry.

Applications (10 copies) must be received no later than February 16, 1979 by the Academic Registrar ( ), University of London, Senate House, London WC1E 7HU, from whom further particulars should first be obtained. 8784(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the post of postdoctoral Senior Research Assistant in the Department of Biochemistry. The post is funded by a grant from the North West Cancer Research Fund to study the hormone responsiveness of a line of human breast cancer cells. Experience of tissue culture would be an advantage.

The initial salary will be within the range £3,883 to £4,882 per annum (Senior Research Assistant 1A). The appointment will be for one year in the first instance.

Applications, together with the names of two referees, should be received not later than January 12, 1979, by The Registrar, The University, Liverpool L69 3BX. Quote Ref. RV/422/N. 8833(A)

UNIVERSITY OF SURREY

DEPARTMENT OF METALLURGY AND MATERIALS TECHNOLOGY

Applications are invited for the post of Redland Research Fellow tenable in the Department of Metallurgy and Materials Technology at the University of Surrey.

The research programme carried out by the Fellow will relate closely to the research and development interests of Redland Limited, and the Fellow normally carries out a number of subsidiary investigations requested by the Company's Research and Development Centre. The post affords an excellent opportunity for the incumbent to familiarise himself with industrial research and to make the transition from academic to the industrial environment.

Applicants should have a Ph.D. or equivalent experience in some area of material science, or solid state chemistry. Preference may be given to candidates with interests or experience in silicate chemistry, Portland cement and ceramics. In view of the nature of the appointment, candidates having the ability to formulate new experimental approaches to unresolved problems would be preferred. The Fellow appointed will undertake research in materials of interest in construction and building and the main concern will be with property structure relationships of interest in developing new or improved materials.

The appointment would be made for two years and can be renewable.

Remuneration will depend on age and experience and will be in the range £3,883 to £4,382 (under review), on scales related to the University lecturer scales. Applications, from men and women, in the form of a curriculum vitae, with the names and addresses of three referees, should be sent to:

Professor J. E. Bailey,
Department of Metallurgy and
Materials Technology,
University of Surrey,
Guildford,
Surrey GU2 5XH 8791(A)

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOCHEMISTRY

RESEARCH TECHNICIAN

(Grade 3)

required for Monoclonal Antibody Production and Biochemical Investigations of Erythrocyte Proteins, particularly those affected by Heredity Spherocytosis, to work in a new well-equipped reserch group investigating cell membrane diseases. A sound biochemical background and previous experience in tissue culture, protein chemistry, electrophoresis and/or chromatography are disirable.

The appointment is from January 1, 1979 initially until July 31, but extendable subject to continuing support from granting agencies, at a starting salary of £3,153 (under review) including London Weighting.

Write or telephone soon for an application form to the Departmental Superintendent, Biochemistry Department, Imperial College, London SW7 2AZ. Tel: 01-589 5111 Ext. 1119, quoting reference DMS RT3.

8713(A)

POSTDOCTORAL POSITION

(funded by the
Cancer Research Campaign)

available for a 3-year study of the role of proteinase inhibitors in the prevention of tumour invasion. A background in cancer research or cell biology would be advantageous, but not essential.

Salary on M.R.C. Scale II, up to £4,631 p.a.

Applications, with curriculum vitae and the names of two referees should be sent to:

Administrative Secretary,
Strangeways Research Laboratory,
Wort's Causeway,
Cambridge. 8845(A)

UNIVERSITY OF RHODESIA
School of Agriculture
DEPARTMENT OF LAND MANAGEMENT

TWO LECTURESHIP vacancies, one in SOIL CHEMISTRY and one in SOIL PHYSICS, exist in the newly-established Department of Land Management, School of Agriculture and applications are invited for these posts.

Depending on qualifications and experience appointments will be made at Lecturer or Senior Lecturer level.

SALARY SCALES (approx sterling equivalent) Senior Lecturer, £7,609 to £10,059, Lecturer Grade I, £6,902 to £7,942, Lecturer Grade II, £4,260 to £6,681

Both Permanent Pensionable Terms and Short-term Contracts are available

Further particulars and application procedure from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Closing date for receipt of applications: JANUARY 15, 1979

British subjects considering applying for posts in Rhodesia are urged to consult the Foreign and Commonwealth Office (telephone 01-233 8727) or their nearest British Consular Office.

8857(A)

COMMONWEALTH AGRICULTURAL BUREAUX
Vacancy for
SCIENTIFIC INFORMATION OFFICER
at the
Commonwealth Bureau of Plant Breeding and Genetics

Department of Applied Biology
Cambridge CB2 3DX

Duties include scanning the world literature dealing with the breeding, genetics and cytology of economic plants, preparing abstracts for publication in **Plant Breeding Abstracts**, and answering enquiries within this field

Qualifications. A degree either in botany, agriculture or genetics or in German is required Applicants combining scientific and linguistic aptitudes will receive prior consideration. Capability to read foreign languages other than German will count as additional qualification Ability to write good English is essential.

Salary. The salary is in the scale £2,839 to £5,448 per annum plus a compensatory allowance (taxable but not superannuable) of 3.3% of basic salary to offset personal contribution to superannuation scheme. Starting salary according to qualifications and experience Promotion to higher scales on merit

Application forms and full particulars from Executive Director, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN

Closing date for applications: January 15, 1979 8807(A)

RESEARCH GRADUATE

to undertake studies in relation to human myeloma cultures and their influences on antibody formation etc Prior experience of tissue culture, lymphocyte culture or bone marrow work is preferred. Excellent opportunity for a Ph D thesis (postdoctoral applicants will only be considered if they already have this experience) Salary Whitley A Scale according to experience. Applications, with 2 referees, to Professor J. R Hobbs, Department of Chemical Pathology, 17 Page Street, London SW1P 2AR, by December 18, 1978 8809(A)

UNIVERSITY OF OXFORD, Department of Forestry. A Research Officer is required for a three-year project in Honduras concerned with the ecology and regeneration of Central American pines. Candidates with an appropriate degree and research experience should apply to the Administrator, Department of Forestry, Commonwealth Forestry Institute, South Parks Road, Oxford OX1 3RB before December 31, 1978, from whom further particulars may be obtained A knowledge of the country and some fluency in Spanish would be desirable Salary on University Scale £3,384 to £8,257 (under review) according to age and experience. 8839(A)

PRINCETON UNIVERSITY
DEPARTMENT OF BIOLOGY

Seeks applicants for the position of.

ASSISTANT PROFESSOR

in the field of population biology. Preferential consideration will be given to candidates whose interests overlap and complement those of existing members of Princeton's population biology group Gould, Horn, May and Terborgh Appropriate areas are. the ecology and adaptive behaviour of animals and plants, population dynamics, sociobiology and biogeography Field experience and a wide ranging interest in organisms are desirable qualifications Evidence of exceptional promise in research is essential Teaching duties will consist of one undergraduate and one graduate level course and counselling students in research. Salary competitive, to be negotiated. Applicants should send a curriculum vitae, a statement of research experience and interests, and three letters of recommendation to. Dr John Terborgh, Department of Biology, Princeton University, Princeton, New Jersey, 08540

Equal Employment Opportunity/Affirmative Action Employer, M/F

8783(A)

UNIVERSITY OF WASHINGTON
CHAIRMAN
DEPARTMENT OF GEOLOGICAL SCIENCES

The College of Arts and Sciences invites applications for the position of Chairman of the Department of Geological Sciences The department has a broad teaching and research program leading to B.S., M.S and Ph.D degrees Twenty faculty members supervise the studies of 70 graduate and 140 undergraduate students.

Extensive facilities are available for research in Crystallography, Isotope Geology, Petrology, Quaternary Geology, Remote Sensing and Lunar Geology, and Structural Geology.

Applicants should submit their curriculum vitae, list of publications and names of four references to Professor Dean A McManus, Chairman of the Search Committee, University of Washington, WB-10, Seattle, Washington 98195.

An Equal Opportunity Affirmative Action Employer 8781(A)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF CHEMISTRY
RESEARCH IN RAMAN SPECTROSCOPY AND ELECTROCHEMISTRY

Recently it has become possible to study the molecular processes involved in electrochemical reactions by making *In situ* Raman spectroscopic observations The pioneer work in this field was carried out in this Department, and an expansion of the effort is planned. We wish to appoint two postdoctoral research fellows as soon as possible

Applications are invited from researchers experienced in vibrational spectroscopy and/or electrochemistry Salary on grade 1A (£3,883 to £6,555)

Further details can be obtained from Dr P. J. Hendra or Professor M Fleischmann on 0703 559122, ext 597 or 371, or by writing to the Department of Chemistry, The University, Southampton SO9 5NH Please quote ref. MH1/2/Na. 8789(A)

THAI NATIONALS

Faculty positions available for graduates in biochemistry or related fields. Good opportunity to join a young dynamic department with active programs in research (currently: nutrition, reproduction and tropical diseases) and teaching (medical and postgraduate students) Special consideration to those trained in biotechnology, cancer and endocrinology. Send curriculum vitae to **Chairman, Department of Biochemistry, Faculty of Science, Mahidol University, Rama VI Rd., Bangkok. Thailand.**

8717(A)

We have an immediate vacancy in the London office for a

PHYSICAL SCIENTIST

preferably one who has some postdoctoral experience. He or she would be engaged largely in the assessment of scientific manuscripts submitted for publication but would probably also help with other sections of the journal. Experience in the earth, ocean or atmospheric sciences would be an advantage.

Write to:
The Editor
NATURE
MACMILLAN JOURNALS LIMITED
4 Little Essex Street, London WC2R 3LF
enclosing a curriculum vitae and any other material you consider relevant.

8661(A)

THE AUSTRALIAN NATIONAL UNIVERSITY
Research School of Chemistry
RESEARCH FELLOWS

Applications are invited for appointment as postdoctoral Research Fellow in the Research School of Chemistry. Appointments will be for two or three years and may be made in any area of chemistry Current major research interests in the School are:-

Organic Chemistry: (Professor A. J. Birch, Mr R. W. Rickards, Dr J. K MacLeod, Dr L. N Mander) including organic chemistry of biologically active compounds; synthetic organic chemistry, organometallic chemistry in organic synthesis, metal-ammonia reduction studies.

Inorganic Chemistry: (Professor A M. Sargeson, Dr M. A Bennett, Professor B G. Hyde (to arrive)): including synthesis, structure and reaction mechanisms of transition metal complexes; biomimetic chemistry and organotransition metal chemistry, solid state inorganic chemistry

Physical and Theoretical Chemistry: (Professor D P Craig, Dr J. Ferguson, Dr R Bramley, Dr T. R Welberry). including molecular and crystal theory, solid state spectroscopy; photophysics, disordered materials

Theoretical Organic Chemistry Dr L. Radom.

Mass Spectrometry: Dr J. K MacLeod: organic and biological applications, ICR spectrometry

X-ray Crystallography: Dr G B Robertson.

Analytical Chemistry: Miss B J Stevenson.

Appointments will be made primarily within these research groups, but proposals for independent research in areas which complement existing programmes will also be considered.

The School is non-departmental and is well equipped to contemporary standards

Salary on appointment will be in accordance with qualifications and experience within the range of $A15,179 to $19,813 per annum, Australian currency. Current exchange rates $A1 : 58NP : $US1.14

Reasonable travel expenses are paid and assistance with housing is given for an appointee from outside Canberra. Superannuation benefits are available

The University reserves the right not to make an appointment or to make an appointment by invitation at any time

There is no application form Applicants should supply to the undersigned by **February 23, 1979** a curriculum vitae, list of publications and statement of research interests, together with two passport-sized photographs, the names and addresses of three academic referees and the probable date on which the postdoctoral Research Fellowship, if awarded, could be taken up.

G E. Dicker,
Academic Registrar,
P.O Box 4,
Canberra, ACT, 2600,
Australia

8832(A)

City Hospital

BASIC GRADE CYTOGENETICISTS

Applications are invited for two posts as Basic Grade Cytogeneticists in the South Trent Sub Regional Cytogenetics Service.

Applicants should have an Honours Degree in Genetics or some other Biological Science.

Preference will be given to candidates with previous experience in diagnostic chromosome analysis or tissue culture. Whitley Council Salary and conditions of service.

Further details and application forms available from: Personnel Services, Valebrook House, Sherwood Hospital, Hucknall Road, Nottingham. Telephone: (0602) 625459 anytime.

Closing date: 5th January 1979. **8824(A)**

NORTH NOTTINGHAM HEALTH DISTRICT (T)

Nottinghamshire Area Health Authority (Teaching)

University College, Galway, Ireland

RESEARCH ASSISTANT

Applications are invited from graduates in Physiology, Biochemistry or allied subjects to study the metabolism of glutamine in isolated segments of kidney tubules. The research is supported by the Medical Research Council of Ireland.

Applications should be sent to: Professor D. J. O'Donovan, Physiology Department, University College, Galway, Ireland, **not later than December 18, 1978.** 8797(A)

CHARING CROSS HOSPITAL MEDICAL SCHOOL

(University of London)

RESEARCH TECHNICIAN

required to work on the development of methods for diagnosis and monitoring of patients with cancer. Experience in protein chemistry or immunology would be an advantage. Post vacant January 1, and the appointment will be for 21 months.

Salary range: £3,261 to £4,680 plus £354 London Allowance.

Further details obtainable from Dr R. Begent, tel: 01-748 2040, extn 2087, and application forms from The Secretary, Charing Cross Hospital Medical School, The Reynolds Building, St Dunstan's Road, London W6 8RP. (Ref: 450/1.) 8850(A)

UNIVERSITY OF OXFORD UNIVERSITY LECTURESHIP IN ELEMENTARY PARTICLE PHYSICS

Applications are invited for the above post. Stipend according to age on the scale (under review) £3,883 to £8,257. The successful candidate may be offered a tutorial fellowship by Hertford College (for either a man or a woman). Details of both appointments may be obtained from:

Professor K. W. Allen, Department of Nuclear Physics, Keble Road, Oxford OX1 3RH, to whom completed applications (one typed copy) and the names of 3 referees should be sent by February 15, 1979. 8810(A)

McGILL UNIVERSITY

Faculty of Medicine

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

DEVELOPMENTAL PHARMACOLOGY

Applications are invited from a senior faculty position from individuals interested in directing a multidisciplinary group in this area of research. The group is guaranteed adequate research space and facilities, a generous operating budget and sufficient monies for clinical and non-clinical investigators as well as support personnel. Curriculum vitae, bibliography, résumé of current research activity and interest and names of three referees should be sent to Dr R. I. Ogilvie, Chairman, Department of Pharmacology and Therapeutics, McGill University, 3655 Drummond Street, Montreal, Quebec, Canada H3G 1Y6. 8820(A)

POSITION OPEN

Postdoctoral position available immediately for individual with background in biochemistry or cellular genetics. Studies involve somatic cell genetics, purine metabolism, nucleic acid synthesis and recombinant DNA technology. Salary $11,000. Contact: Dr Milton Taylor, Department of Biology, Indiana University, Bloomington, IN 47402. An Equal Opportunity/Affirmative Action Employer. 8821(A)

UNIVERSITY OF THE SOUTH PACIFIC

Applications are invited for the post of

UNIVERSITY FELLOW

in the INSTITUTE OF MARINE RESOURCES (Post 78/75). This vacancy exists at the newly established Atoll Research Unit. This unit is to be located on Tarawa, in the Gilbert Islands. The appointee will be initially required to develop and carry out where practicable the applied research and advisory and educational programmes of the Unit, in consultation with the director of I.M.R. The most immediate project to be undertaken by the appointee is a survey of Tarawa lagoon and subsequent monitoring of any existing pollution indices. The appointment will be made at lecturer or senior lecturer level. Housing and some laboratory facilities are available. An appropriate vehicle for transportation on Tarawa will be provided. Salary Scales: Senior Lecturer F$11,178 to F$13,209 per annum; Lecturer F$7,640 to F$10,808 per annum (salary scales are curerntly under review). (£1 sterling=F$1.62.) The British Government may supplement salary in range £1,842 to £2,796 per annum (sterling) for married appointee or £444 to £1,164 per annum (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. In addition, the University provides 15% gratuity, superannuation contribution, appointment allowance, partly furnished accommodation at maximum rental of 12½% of salary (under review), family passages. Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Registrar, University of the South Pacific, P.O. Box 1168, Suva, Fiji, by January 22, 1979 quoting post reference number. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 8855(A)

THE UNIVERSITY OF MANCHESTER

TEMPORARY LECTURER IN RADIOCHEMISTRY

Applications are invited for this one-year post starting as soon as possible.

Salary range £3,883 to £4,631 p.a.

The radiochemistry group in the Department of Chemistry consists of another member of staff, two post-doctoral fellows and eight postgraduate students. There is a wide range of research topics including nuclear reaction studies, activation analysis and radiation effects in solids. The appointee would play an active role in the teaching and research activities of the group.

Particulars and application forms (returnable by January 6) from the Registrar, The University, Manchester M13 9PL. Quote ref: 270/78/N. 8843(A)

UNIVERSITY OF BRISTOL

DEPARTMENT OF BACTERIOLOGY

VIROLOGY RESEARCH ASSISTANT—RANGE 1A

A vacancy exists for a Research Assistant for a period of 21 months to undertake investigation into the RNA biochemistry of measles virus in chronic infections. The preferred applicant would be a postdoctoral biochemistry graduate with particular experiences of nucleic acid (especially RNA) techniques.

The post is M.R.C. funded and the commencing salary will be up to £4,133 per annum (scale under review).

Applications, citing two referees, should be forwarded to Dr L. W. Greenham, Department of Bacteriology, University of Bristol BS8 1TD. 8846(A)

THE WEST OF SCOTLAND AGRICULTURAL COLLEGE AGRONOMY SPECIALIST

Applications are invited for the above post in Agronomy Department tenable for three years, within a group responsible for the evaluation of sewage sludge as a fertiliser for grassland. The duties will be primarily concerned with overseeing the planning and conduct of field performance trials in which comparisons are made between sludge and conventional fertilisers. Duties would also include literature review, preparation of data for publication and the writing of advisory literature.

Candidates should have an honours degree or equivalent qualification in grassland/crop production or related discipline, or a higher degree, and a special interest in experimental work. The appointee will be based at Auchincruive but appreciable travel is involved and a current driving licence is essential.

The starting salary will, according to age, qualifications and experience, be on one or other of the following scales:-

Grade IV—£2,998 (Honours—1st or 2nd Class—£3,167) rising to £3,924.

Grade III—£4,178 rising to £6,898.

Conditions of appointment and application forms are available from the Secretary, The West of Scotland Agricultural College, Auchincruive, Ayr KA6 5HW, with whom applications should be lodged not later than January 5, 1979. 8837(A)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF BIOMEDICAL PHYSICS AND BIOENGINEERING

RADIATION PROTECTION OFFICER

Applications are invited from PHYSICISTS for the above post carrying duties throughout the University and with certain service commitments to hospitals in the Grampian, Orkney and Shetland areas. Graduate technical and secretarial assistance is provided. The successful candidate will be expected and encouraged to participate in the general activities of the Department including teaching and research.

Salary on Lecturer Scale £3,883 to £7,754 per annum, with appropriate placing. Superannuation benefits.

Further particulars from The Secretary, The University, Aberdeen with whom applications (2 copies) should be lodged by January 12, 1979. 8836(A)

UNIVERSITY OF OXFORD UNIVERSITY LECTURESHIP IN BIOCHEMISTRY

Applications are invited for the above post for appointment from October 1, 1979, Stipend according to age on the scale £3,883 to £8,257 p.a. Other things being equal, preference may be given to candidates with research interests in nucleic acid chemistry, carbohydrate chemistry, immunology, or bioenergetics. The successful candidate may be offered appointment as an official fellow and tutor at St Catherine's College (open to men and women). Further particulars of both the University and college posts may be obtained from Professor R. R. Porter, Department of Biochemistry, South Parks Road, Oxford OX1 3QU. Applications for the lectureship (eight typed copies, one from overseas candidates) including the names of three referees should be sent to Professor Porter by January 31, 1979. Applications for the college fellowship should be sent to The Master, St Catherine's College, Oxford OX1 3UJ by the same date. 8811(A)

EDUCATION OFFICER

required by the Sussex Trust for Nature Conservation for development of educational activities, including servicing of Woods Mill Educational Centre. Starting salary £3,300 to £3,500. Car ownership essential. Details and application form from The Administrative Officer, S.T.N.C., Woods Mill, Henfield, West Sussex. 8808(A)

LIVERPOOL POLYTECHNIC
DEPARTMENT OF BIOLOGY
RESEARCH ON DEVELOPMENT OF CONTAMINATED LAND
(3 posts)

This is a long-term contract undertaken for and funded by the Department of the Environment to establish optimum soil cover and ameliorant treatments for reducing the uptake of cadium and other trace metals by vegetables and soft fruits from contaminated land intended for housing. The Project is based in Liverpool, with a permanent research site at a former Sewage Farm near Leicester.

In conjunction with the establishment of the Project, three posts are offered: —

1. POSTDOCTORAL RESEARCH FELLOW

To assist in the overall direction and formulation of the study, including day-to-day control of the research work; the assessment of analytical and other data and the preparation of regular Project reports. The appointee will be responsible for the analysis and interpretation of materials collected from the main experimental plots. He/she will also be required to liaise effectively with the Site Officer, and to assist the Project Director in plotting the overall course of the study. Frequent visits to Leicester will be involved, but the appointee will be based in Liverpool. A current driving licence is essential.

QUALIFICATIONS: The successful candidate should possess a Ph.D. in an appropriate botanical or agricultural field, and should, if possible, have previous experience in field trials.

Salary scale: £4,101 to £6,558 p.a.

2. SITE OFFICER

The appointee will be responsible for the day-to-day management of the permanent research site, and will be based full-time in Leicester. The duties will include planting, sampling, plot maintenance and supervision of other long-term experiments and ancillary staff. The Site Officer will also be responsible for liaison with contractors during the establishment phase of the research station. It may be possible for a suitable person to register for a higher degree during the course of the Project. A current driving licence is an advantage.

QUALIFICATIONS: A degree or equivalent in Agriculture, Botany, Horticulture or a related field. Some experience of management is desirable.

Salary scale: £3,192 to £5,334 p.a.

3. ANALYST/TECHNICIAN

To carry out analysis in connection with the Project using mainly atomic absorption techniques. Based in Liverpool, but may be required to assist at research site at Leicester in sampling, etc., to meet seasonal peak work-loads.

QUALIFICATIONS: An appropriate degree or related qualification and relevant analytical experience.

Salary scale: £3,192 to £5,334 p.a.

Application forms and further particulars are available from the Personnel Office, Liverpool Polytechnic, Richmond House, 1 Rumford Place, Liverpool L3 9RH. Tel: 051-227 5581, extn 43.

Please quote: Reference LP/250.

Closing date for applications is January 12, 1979. 8862(A)

BEATSON INSTITUTE FOR CANCER RESEARCH
SCIENTIST

Postdoctoral scientist required with immunological experience to join a new three year project using the Hybridoma technique for raising monoclonal anti-bodies to analyse red blood cell development. Please apply to: Dr P. R. Harrison, The Beatson Institute, Garscube Estate, Switchback Road, Bearsden, Glasgow. 8865(A)

NEW ZEALAND
University of Canterbury Christchurch

Applications are invited for the following position:

LECTURER IN EXTENSION STUDIES

Applicants should possess a higher degree in Science. The appointee will be required to carry out teaching, administrative and organisational responsibilities with special reference to an extension programme in science.

Applications close on **January 15, 1979.**

The salary for a Lecturer is on a scale from NZ$11,894 to $14,488 per annum.

Particulars, including information on travel and removal allowances, study leave, housing and superannuation, may be obtained from the Association of Comonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. 8830(A)

UNIVERSITY OF LONDON
INSTITUTE OF ARCHAEOLOGY
BOTANIST

required with knowledge of plant taxonomy and anatomy to work on botanical material from archaeological excavations in England. A degree in a relevant subject, familiarity with pollen and seeds and previous archaeological experience would be advantageous.

SKELETAL BIOLOGIST

required for studies on excavated early British human skeletal samples. Candidates should hold an appropriate degree and preferably have some postgraduate experience in this field.

Both posts on scale for Research and Analogous Staff £3,410 to £4,892 per annum plus £450 per annum London Allowance. Appointments initially for three years from February 1, 1979, or as soon as possible thereafter. Further details from the Secretary, Institute of Archaeology, 31-34 Gordon Square, London WC1H 0PY, telephone 01-387 6052. Closing date for applications January 5, 1979. 8829(A)

UNIVERSITY OF ASTON IN BIRMINGHAM
DEPARTMENT OF MATHEMATICS
LECTURESHIP

Applications are invited for appointment as a lecturer in the Department of Mathematics. In addition to the usual teaching and examining responsibilities of this post, the appointee will be expected to participate in the research programme and administration of the Earth Satellite Research Unit. This Unit is funded by S.R.C. and operates two Hewitt cameras, one installed near Birmingham and the other to be sited in Australia.

Research experience in classical mechanisms and computing or in other relevant areas is required.

Commencing salary will be within the range £3,883 to £5,842 per annum according to age and qualifications) on a scale rising by annual increments to £7,754 per annum (currently under review).

Application forms and further particulars may be obtained from the Staff Officer (Ref. 322/6) University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET (Tel. 021-359 3611 Ext. 201) to whom applications should be forwarded not later than January 1, 1979. 8796(A)

BIOLOGY TEACHING OVERSEAS

RESEARCH–TEACHING positions available beginning Fall 1979 in such specialties as Ecology, Plant Physiology, Developmental Biology, Invertebrate Zoology. Ph.D. required, rank and salary depend on experience and qualifications. Usual contract is for 3 years with RT travel and other fringe benefits.

Curriculum vitae and letters of recommendation should be airmailed to: Dr Samir Deeb, Biology Department, American University of Beirut, Beirut, Lebanon.

Application review will begin March 1, 1979. 8842(A)

THE BRITISH COUNCIL

invites applications for the post of:

Lecturer in Plant Genetics
(Sri Lanka)

UNIVERSITY OF SRI LANKA, JAFFNA CAMPUS,

to plan and conduct undergraduate and advanced courses in Genetics and to guide postgraduate research. Candidates should hold a Ph.D., preferably in Plant Genetics or related field and have 2–3 years' teaching experience.
Salary: £5,681 to £7,707.

Benefits: Personal allowance, free furnished accommodation and other benefits. 2-year contract. Return fares are paid. Local contracts are guaranteed by the British Council.
Please write briefly stating qualifications and length of appropriate experience, quoting reference 78 PU 102 and title of post, for further details and application form to:

The British Council (Appointments)

65 Davies Street, London W1Y 2AA

8840(A)

UNIVERSITY COLLEGE GALWAY

DEPARTMENT OF MICROBIOLOGY

RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant commencing on January 1, 1979. This post, funded by the Medical Research Council of Ireland, will involve an investigation of mutagenesis in *E. coli.*

Applicants should hold a Degree in microbiology and should write immediately to:

Dr E. Farrell, Department of Microbiology,
University College Galway, Ireland

8860(A)

UNIVERSITY OF STRATHCLYDE

Applications are invited for a

POSTDOCTORAL RESEARCH FELLOW
or a
GRADUATE RESEARCH ASSISTANT

in Organic Chemistry in the Department of Pure and Applied Chemistry

The work will involve an investigation of chemotherapeutic agents based on studies of the biosynthesis of dihydrofolate. The appointee will have the opportunity to work in the areas of synthetic organic chemistry and enzyme chemistry and to collaborate with scientists in a major pharmaceutical company. The Graduate Research Assistant may be eligible to pursue research leading to a higher degree.

Salary scales on the national salary structure for research and analogous staff: Postdoctoral Research Fellow, Range 1A, commencing salary within the scale £3,883 to £4,882; Graduate Research Assistant, Range 1B, commencing salary within the scale £3,384 to £4,382. Superannuation benefit.

The appointment will date from January 1, 1979, or such other date as may be arranged and will be tenable for up to a period of three years.

Applications with curriculum vitae and names and addresses of two referees (quoting R47/78) should be sent as soon as possible to Professor H. C. S. Wood, Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow G1 1XL, from whom further particulars may be **obtained.** 8859(A)

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)

SENIOR LECTURER/LECTURER IN MEDICAL PHYSICS
(Physiological/Clinical Measurement)

Applications are invited for the above newly established post, preferably at Senior Lecturer level. An honours degree in physics (or related subject), together with a higher degree is required, together with substantial knowledge and experience of electronics, preferably in the medical field. The duties will involve:

(i) Participation in the Research and Development work of Pre-Clinical and Clinical College Departments, including work on equipment associated with patient care;
(ii) Initiation of basic research;
(iii) Undergraduate and Postgraduate teaching in association with other Departments where required;
(iv) Liaison with the Physics Departments of the London Hospital.

Salary according to qualifications and experience in the range: Senior Lecturer, £7,506 to £9,263 plus £450 London Allowance p.a.; Lecturer, £4,382 to £7,754 plus £450 London Allowance p.a., together with superannuation under U.S.S. or N.H.S.

Further information available from Professor M. Braden, tel: 01-247 0644, extn 80. Applications, giving full details of qualifications and experience, together with the names of two referees, should be sent to The Secretary, The London Hospital Medical College, Turner Street, London E1 2AD, within **21 days.** 8861(A)

KINGSTON POLYTECHNIC
SCHOOL OF CHEMICAL AND PHYSICAL SCIENCES
SENIOR TECHNICIAN

To be responsible for a Life Science laboratory, used for undergraduate teaching, research and project work. Duties will include microbiology, plant studies and tissue culture work, plus experimental development and investigational work. An important job requiring someone with initiative and enthusiasm. Further details on application.

T3/4 grade £4,017 to £4,917 inclusive.

Application forms from Assistant Registrar (Personnel), Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. 01-549 1366.
8825(A)

UNIVERSITY COLLEGE CARDIFF

Applications are invited for the post of:

LECTURER

in the DEPARTMENT OF BIOCHEMISTRY.

Salary range: £3,883 to £7,754. Duties to commence as soon as possible.

Applications (2 copies), together with the names and addresses of two referees, should be forwarded to the Vice-Principal (Administration) and Registrar, University College, P.O. Box 78, Cardiff CF1 1XL, from whom further particulars may be obtained. Closing January 8, 1979. Please quote reference 1660.
8817(A)

CONGRESS

To mark its Centennial in 1980, The Graduate School of Arts and Sciences of Columbia University in the City of New York will convoke an International Congress meeting on

THE LANGUAGES OF KNOWLEDGE AND OF INQUIRY

concerned with the means whereby the various sciences, social sciences, and humanities pursue their inquiries and conceptualize and transmit their results.

Interested parties should write to:

Office of the Dean
The Graduate School of Arts and Sciences
Columbia University
New York, NY 10027
8819(Q)

SYMPOSIA

ANNOUNCING THE SECOND INTERNATIONAL SYMPOSIUM ON INVERTEBRATE REPRODUCTION

WHEN: AUGUST 27 to AUGUST 31, 1979

WHERE: UNIVERSITY OF CALIFORNIA, DAVIS

For registration material, contact (by January 15, 1979):

Mrs Carolyn Norlyn
ISIR Conference Coordinator
4475 Chemistry Annex
University of California
Davis, Ca. 95616 U.S.A.

Monday, August 27: Physiology of gamete activation and interaction. Chairs: R. A. Leopold, USDA, Fargo, N. Dakota; V. D. Vacquier, University of California, San Diego.

Tuesday, August 28: Gonad-produced hormones. Chairs: A. Schuetz, Johns Hopkins University, Baltimore, Md.; K. G. Davey, York University, Ontario, Canada.

Wednesday, August 29: (informal meetings/workshops).

Thursday, August 30: Environmental synchronisation of reproduction. Chairs: J. Pearse, University of California, Santa Cruz; R. M. Woollacott, Harvard University, Cambridge, Mass.

Friday, August 31: Artificial manipulation of reproduction. Chairs: D. Morse, University of California, Santa Barbara; L. L. Sower, USDA, Forest Service, Corvallis, Oregon.

Symposiums will be held in the mornings, and afternoons will be devoted to contributed papers.
8854(M)

LECTURES

1st FEBS LECTURE TOUR

A new Lectureship Programme has been started by the Advanced Courses Committee of the Federation of European Biochemical Societies. The 1st FEBS Lecture Tour will take place in 1979.

GARY FELSENFELD

(Bethesda, Md)

will lecture on

"Nucleosome Architecture and Chromatin Function"

as follows:

February 1,	London (King's College Department of Biophysics)
February 1,	Cambridge (M.R.C. Laboratory of Molecular Biology)
February 5,	Orléans (Centre de Biophysique Moléculaire)
February 6,	Paris (Institut de Recherche en Biologie Moléculaire)
February 8,	Munich (Institut für Physiologische Chemie, Physikalische Biochemie, und Zellbiologie)
February 9,	Heidelberg (European Molecular Biology Laboratory)
February 12,	Strasbourg (Laboratoire de Génètique Moléculaire des Eucaryotes)
February 14,	Barcelona (Depto. de Quimica Macromolecular)

8799(K)

ASSISTANTSHIPS

BRIGHTON POLYTECHNIC
Faculty of Natural and Life Sciences
DEPARTMENT OF PHARMACY
RESEARCH ASSISTANTSHIP

Applications are invited from honours graduates in pharmacy, biochemistry or related disciplines, to pursue research in one of the following areas:—

1. The interaction of drugs with mucus;
2. The effects of biopolymers on drug absorption;
3. The release of drugs from polymeric systems.

Salary scale: £2,613 by £87 (2) to £2,787.

Details of post and application form from Administrative Officer, Faculty of Natural and Life Sciences, Brighton Polytechnic, Moulsecoomb, BRIGHTON BN2 4GJ, to whom completed forms should be returned by January 3, 1979
8849(P)

IMPERIAL COLLEGE
DEPARTMENT OF ZOOLOGY

Applications are invited for a postdoctoral Research Assistantship tenable at the College Field Station for work for three years on a Medical Research Council sponsored project on the sexual development of malarial parasites. The successful applicant will be expected to develop new methods for the culture and purification of the parasite, and to study the biochemistry/physiology of the parasite plasmalemma. Experience in the fields of cell fractionation of membrane chemistry would be an advantage.

Salary in the range £3,883 to £4,631 on the Research and Analogous Staff scale, plus U.S.S.

Applications with the names of two referees in writing by December 15, to Dr R. E. Sinden, Imperial College Field Station, Ashurst Lodge, Ascot, **Berks.**
8604(P)

ASSOCIATESHIP

HERIOT-WATT UNIVERSITY
DEPARTMENT OF PHYSICS
POCTDOCTORAL RESEARCH ASSOCIATESHIP IN SOLID STATE THEORY

Applications are invited for an S.R.C. Postdoctoral Research Associateship, for studies of Anderson localisation and other topics in the theory of disordered solids, in association with Dr D. Weaire. The starting date should be no later than October 1, 1979.

Informal enquiries can be addressed to Dr Weaire.

Initial salary will be in the range £3,883 to £4,382 p.a. (under review) of Range 1A.

Application forms are obtainable from the Staff Officer, Heriot-Watt University, Chambers Street, Edinburgh EH1 1HX. Ref. No. 22/78.
8841(O)

FELLOWSHIPS

UNIVERSITY OF NOTTINGHAM
FACULTY OF AGRICULTURAL SCIENCE
Readvertisement
RESEARCH FELLOWSHIPS (PHYSICS/ENGINEERING)

Two fellowships are available for projects supported by the A.R.C. The first is concerned with the physics and economics of very low cost solar energy collectors for use in crop drying and the successful candidate will work with Dr J. A. Clark and Mr B. Wilton. The second project, supervised by Professor J. L. Monteith, will examine the feasibility of assessing the growth rate of crops from the spectral composition of reflected radiation, including measurements from the air.

Both appointments are for three years. Candidates should preferably have a Ph.D. or equivalent experience in an appropriate branch of physics, engineering, or environmental science (salary scale commencing £4,382 per annum at age 26). Applications will also be considered from newly qualified graduates with a good honours degree (salary scale commencing at £3,384 per annum).

Applications with the names of two referees should be submitted to and further details may be obtained from The Secretary, University of Nottingham School of Agriculture, Sutton Bonington, Nr. Loughborough.
8790(E)

The Wellcome Trust

Wellcome Research Fellowships in Veterinary Medicine

Applications are invited for research fellowships in Veterinary Medicine from Veterinary graduates in British Universities who have already had at least three years' post-graduate research experience.

The Fellowships are tenable for up to three years and may be held in any University Department or Research Institute in the United Kingdom concerned with research. Applications must be sponsored by the Head of a Department who is an expert in the field of study chosen by the candidate. Age limit is 32 years.

Salary is according to age: £4,382 per annum at the age of 26, rising to £5,842 per annum at the age of 32. In addition £500 per annum is given to the Head of the Department as a contribution towards the research expenses of the Fellow. In cases where veterinary graduates propose to submit their work for a PhD degree a lower scale will apply.

Wellcome Veterinary Research Training Scholarships

Applications are invited from Veterinary graduates of British Universities (or those who expect to graduate in 1979) for Research Training Scholarships in the United Kingdom.

These scholarships are for training in research techniques and may be held in any University Department or Research Institute in the United Kingdom in which work can be carried out for a PhD. Applications must be sponsored by an expert in the field of study chosen by the candidate.

The Scholarships are tenable for three years subject to satisfactory annual reports, and carry a stipend of £2,752 per annum. In addition the Trustees will pay for all approved College Fees and £500 per annum is given to the Head of the Department as a contribution towards the research expenses of the scholar.

Application forms are available from: Grants Section, The Wellcome Trust, 1 Park Square West, London NW1 4LJ. Tel: 01-486 4902.

Closing date for applications is 14th February, 1979.

8828(E)

Cancer Research Campaign

Post-Doctoral Research Fellowship in Biochemistry

A postdoctoral research fellowship is now available for work at the Microbiological Research Establishment on coupling biologically active proteins to polysaccharides to improve performance in antitumour therapy and for application in diagnostic enzymology. Experience in protein : polysaccharide interaction and enzymology is desirable. The post is tenable for up to three years with a starting salary in the range £4,101 to £4,579.

Research Assistant

An assistant with good academic qualifications is required to support the work of a postdoctoral research scientist on the above project. The post is tenable for up to three years with a starting salary in the range £2,319 to £2,633.

Applications, including a full curriculum vitae and two academic referees should be sent to The Director, Microbiological Research Establishment, Porton, Salisbury, Wiltshire.

Closing date for applications is January 4, 1979.

8803(E)

THE ROYAL SOCIETY
LOCKE RESEARCH FELLOWSHIP
ROSENHEIM RESEARCH FELLOWSHIP

Applications are invited by the Council of the Royal Society for:

(a) A Locke Research Fellowship for research in experimental physiology and pharmacology;

(b) The Rosenheim Research Fellowship established under the will of the late Mrs M. C. Rosenheim for research on the biochemistry of plants and the simpler forms of animal life. Preference will be given to candidates wishing to study the biochemistry of higher plants.

Both appointments are tenable in any appropriate university department or research institution approved by the Council of the Royal Society (the Rosenheim Research Fellowship is restricted to places in Great Britain). Candidates should supply the usual personal details and two referees. Testimonials will not be considered. The subject of the proposed research and where it will be done, together with the name of the head of department, whose consent should first be obtained, must be stated.

The appointments, which will be subject to the Society's general regulations governing research appointments, will be tenable for three years in the first instance from October 1, 1979, and may be renewable for a further two years, such renewal being considered at the end of the second year of tenure. Applicants should preferably be under 33 for the Locke Research Fellowship and under 26 for the Rosenheim Research Fellowship on October 1, 1979.

Stipend scales are as follows (the point of entry will be determined by Council), and superannuation benefits are provided: Locke Research Fellowship (Category C): £6,153 by £240 to £7,113 by £243 to £7,356 per annum; Rosenheim Research Fellowship (Category D): £4,461 by £249 to £5,706 per annum.

Applications should be made on forms to be obtained (stating name of Fellowship) from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, and should be received not later than February 28, 1979.

8848(E)

FELLOWSHIPS continued

UNIVERSITY OF SUSSEX
SENIOR AND JUNIOR RESEARCH FELLOWSHIPS
IN THEORETICAL ASTRONOMY

Applications are invited for S R C.-supported posts of Research Fellow in Theoretical Astronomy, for a period of one or two years, with possible extension to a third. There is one Senior Research Fellowship, with a stipend equivalent to the Reader scale (£7,506 to £9,263 per annum, under review) The junior posts are based on the Research Fellow 1A scale (£3,883 to £6,555 per annum, under review) All posts carry U.S S. benefits Appointments will normally commence on October 1, 1979.

Candidates should propose an independent research programme Further information can be obtained from Professor L Mestel, Astronomy Centre, Physics Building, University of Sussex, Falmer, Brighton BN1 9QH, U.K Applications should arrive by **January 31, 1979.** Applicants should ask up to three referees to send confidential reports, to reach the Astronomy Centre by the same date.

8801(E)

CHRISTIE HOSPITAL & HOLT RADIUM INSTITUTE

Withington, Manchester M20 9BX

PATERSON LABORATORIES

FELLOWSHIP IN CELL BIOLOGY

Fellowship available at postdoctoral level for studies on epithelial proliferation and differentiation control in collaboration with Dr C S Potten Some experience in the cellular pathology of replacing tissues would be an advantage The fellowship (£4,631 per annum) would be tenable from early 1979

Letters of application together with curriculum vitae should be sent to the Director, Professor L G Lajtha, as soon as possible Ref· 78/72

8794(E)

JOHN INNES INSTITUTE POSTDOCTORAL FELLOWSHIP

The use of protoplasts for the interspecific and intergeneric transfer of chromosome fragments.

Work with animal cells has shown that heterokaryons formed by the fusion of somatic cells can be exploited in such a way as to lead to the transfer of chomosome fragments from a donor to a recipient cell We now seek a postdoctoral fellow to explore the possibility of similarly exploiting heterokaryons of plant cells There is considerable expertise in the technology of protoplast production and manipulation within the John Innes Institute where protoplasts are used as vehicles for studying virus infection and multiplication The person appointed will work in collaboration with this group.

Candidates who have a background in molecular biology, genetics or related fields are invited to apply for this fellowship which is for a period of three years and will be funded by the John Innes Charity Salary will be in the scale £4,101 to £5,065 per annum according to experience Superannuation.

Further details are available from Professor R W. Horne, John Innes Institute, Colney Lane, Norwich NR4 7UH

Applications with curriculum vitae, full details and the names of two referees should be sent to the Secretary, John Innes Institute, Colney Lane, Norwich NR4 7UH by February 3, 1979.

8823(E)

THE OPEN UNIVERSITY

Discipline of Earth Sciences Postdoctoral Research Fellow

Applications are invited for a three year postdoctoral research fellowship supported by NERC The PDRA will investigate metamorphic and geochemical changes associated with major shear belts in the Archaean craton of South India The study will be part of a collaborative project with the National Geophysical Research Institute and Geological Survey of India. Previous analytical and field experience in complex Precambrian terrains would be an advantage.

Salary in the range £3,883 to £6,555 p a. with superannuation benefits Appointment is from as soon as possible

Application forms and further particulars are available, by postcard request please, from The Recruitment Office (JD3722/2), The Open University, P O Box 75, Walton Hall, Milton Keynes, MK7 6AL or telephone Milton Keynes 63868 24 hour answering service. Closing date for applications. 20th December, 1978

8822(E)

UNIVERSITY OF SURREY RESEARCH FELLOWSHIP
EMBRITTLEMENT BY LIQUID METALS

There is a vacancy in the field of high temperature surface reactions for work concerned with the embrittlement of steels by liquid zinc The structure, composition and the physical nature of the reacted specimens will be examined using a wide range of analytical techniques including metallography, Electron-probe, E S C A etc.

The post will be suitable for either a postdoctoral Fellow wishing to gain further experience in the field of high temperature surface reactions or a graduate experienced in the field of solid metal-liquid metal surface reactions

Preference will be given to candidates with a lively interest in practical work and an imaginative approach to interpretation. The appointment will be for two years Starting date is visualised as January 1, 1979 or as soon as possible thereafter The salary will be on an appropriate point on a scale range £3,883 to £5,367 per annum

Applications in the form of a curriculum vitae, including the names and addresses of two referees should be sent to Dr J Mackowiak, Department of Metallurgy and Materials Technology, University of Surrey, Guildford, Surrey GU2 5XH

8792(E)

UNIVERSITY OF GLASGOW
DEPARTMENT OF CHEMISTRY
M.R.C POSTDOCTORAL FELLOWSHIP

The above position is available from March 1, 1979, for the determination of the structure of Chloramphenicol acetyl transferase at atomic resolution. Experience in protein crystallography is essential Starting salary will be within Range 1A of the salary scale for Research and Analogous Staff, £3,883 to £4,382, depending on age and experience

Further particulars are available from Dr I D A Swan, Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, to whom applications, together with a curriculum vitae and the names of two referees, should be addressed

In reply please quote Ref No 4325M.

8812(E)

SCHOLARSHIPS

QUEEN ELIZABETH COLLEGE
Kensington
(University of London)
POSTGRADUATE RESEARCH SCHOLARSHIPS
October 1979

Applications are invited for postgraduate research scholarships in science tenable at Queen Elizabeth College Candidates should, by the date of the appointment, have obtained a first class or an upper second class honours degree from a British University, or hold an equivalent qualification and they should have a clear idea of the topic on which they wish to undertake research Appointment will be offered following competitive interview and will be in one of the College departments or units, i e :-

Biochemistry
Biology
Chemistry
Food Science and Nutrition
(including Management Studies)
Mathematics
Microbiology
Physics
Physiology
Computer Unit
Isotope Unit

The maximum value of the basic maintenance award is £1,990 p a (1978/79 rates, currently under review), plus approved fees Certain expenses and allowances are payable in addition where applicable Holders may be asked to undertake demonstrating for which additional payment will be made.

Queen Elizabeth College is a science-based institution with approximately equal numbers of men and women students. Out of a student body of some 1,050, 250 are postgraduates As the departments are all on the same pleasant four-acre site on Campden Hill in Kensington, interdisciplinary contacts are readily established Public transport and parks are nearby Some accommodation is available in College-owned houses

Further particulars and application forms are obtainable from the Academic Registrar, Queen Elizabeth College, Campden Hill Road, London W8 7AH, to whom application forms should be returned by April 1 1979

8802(H)

COURSES

Summer School on
BIOENERGETICS AND THEMODYNAMICS· MODEL SYSTEMS

Synthetic and natural chelates and macrocycles as models for biological and pharmaceutical studies

NATO—Advanced Study Institute
TABIANO/Salsomaggiore—
Parma/Italy
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SCIENTIFIC PROGRAMME

Design and synthesis of ligands Equilibria methods and results Thermochemistry· methods and results Metals, protons and proteins energetics, conformation, complexation Selectivity of macrocycles toward cations and anions Thermodynamics and kinetics in complex formation Bioenergetics of drug-receptor interactions Membranes Calorimetry of biological systems

The School will be of interest to scientists working in the fields of organic chemistry, bio-inorganic chemistry, physical chemistry, biophysics, biochemistry, molecular biology, medicinal chemistry, molecular pharmacology

For information write to
Professor A Braibanti
Faculty of Pharmacy,
Via M D'Azeglio, 85
I-43100 Parma (Italy)
Tel (0521) 35 801
Telex 53327 UNIV PR I

8853(D)

FEBS ADVANCED COURSES

No. 58, "Biomolecular Electron Microscopy"
Ulm, FRG,
29 March—7 April 1979

The Course, organized by A K. Kleinschmidt, is intended to offer information on selected methods of biomolecular structure studies, and laboratory instruction in electron-microscopic methods of macromolecular analysis, to 40 qualified applicants Specialised fields, e.g. electron microscopy of nucleic acids, membrane structure, nucleoprotein particles, immunocytochemistry, lipoproteins and proteoglycans, and autoradiographic procedures and image analysis methods will be included to participants' choice in laboratory practical sessions. Course fee, including accommodation and food; approx DM400 — Applications (b **January** 1979) and info. Dr A K. Kleinschmidt, University of Ulm, Department of Microbiology, Postfach 4066, 7900 Ulm, FRG.

No. 60. "Gene Structure and Formation of RNA of Viruses and Animal Cells"
Israel, 15—21 May 1979

A workshop organized by Y. Aloni and co-sponsored by EMBO. Fee: $280.— Info: Mr Y. Berman, Congress Section, Weizmann Institute of Science, Rehovot, Israel.

No. 62. "Genome Organization and Expression in Higher Plants"
Edinburgh, Scotland,
11–21 July 1979

A course for up to 125 participants Accommodation including ful board, £130 approx Applications (**by 14 February 1979**) and info: Dr C Leaver, Dept of Botany, The King's Buildings, Mayfield Road, Edinburgh, EH9 3JH, Scotland

No. 56. "Membrane Proteins"
Berne, Switzerland,
10–22 September 1979

The course, co-sponsored by the Swiss Committee for Molecular Biology and organized by P Zahler and A Azzi, will consist of 1 week each of lectures and experimental work, and discussions The course will concentrate on specific problems of membrane proteins (solubilization, fractionation, isolation, characterization, reconstitution) Places will be restricted to 80–100 for the lectures and to 40 advanced participants for the experimental part Fees (including registration, accommodation and food)· Sw Fr.700.—for the full course or Sw Fr 340 —for the lecture course only Applications should be addressed **before 1 April, 1979** to: Professor P Zahler, Institut für Biochemie, Universität Bern, Freiestrasse 3, CH-3012 Bern, Switzerland.

No. 57. "Genetic Engineering"
Zagreb, Yugoslavia,
8–24 October 1979

Info: Dr V. Škarić, Institut "Ruđer Bošković", Post. pret 1016, 41001 Zagreb, Yugoslavia.

8800(D)

UNIVERSITY OF BRISTOI
12 MONTHS M Sc. COURSE
BY ADVANCED STUDY AND RESEARCH

are offered in the following subjects

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(Inorganic Chemistry)
Surface Chemistry and Colloids
(Physical Chemistry)
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Mathematical Logic and Foundatio
of Mathematics (Mathematics)
Fluid Mechanics (Mathematics)

All five courses qualify for a quc of S R C. advanced course studei ships Further details of the individi courses may be obtained from t Head of the Department concern University of Bristol BS8 1TH E quiries are also invited for details opportunities for research leading the degree of Ph.D.

8798(D)

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Microbiological Research Establishment

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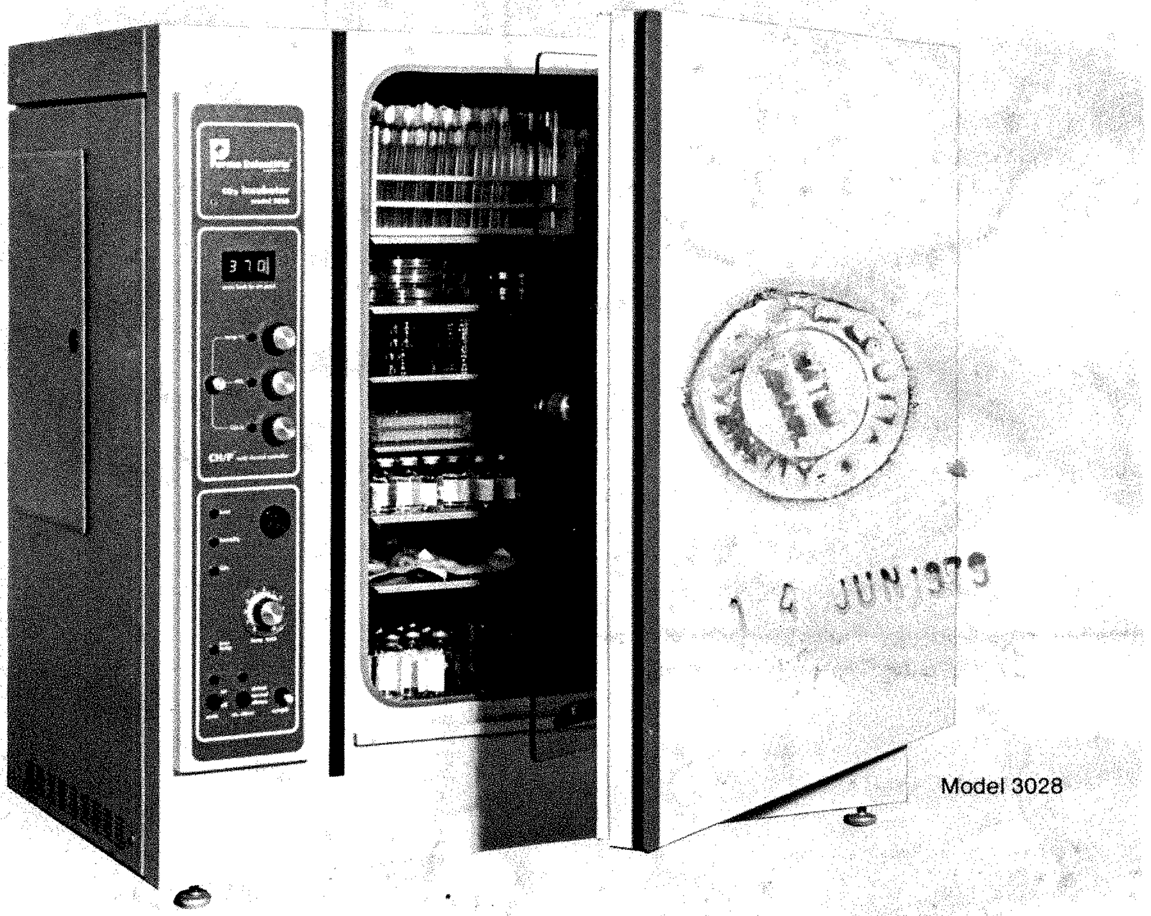

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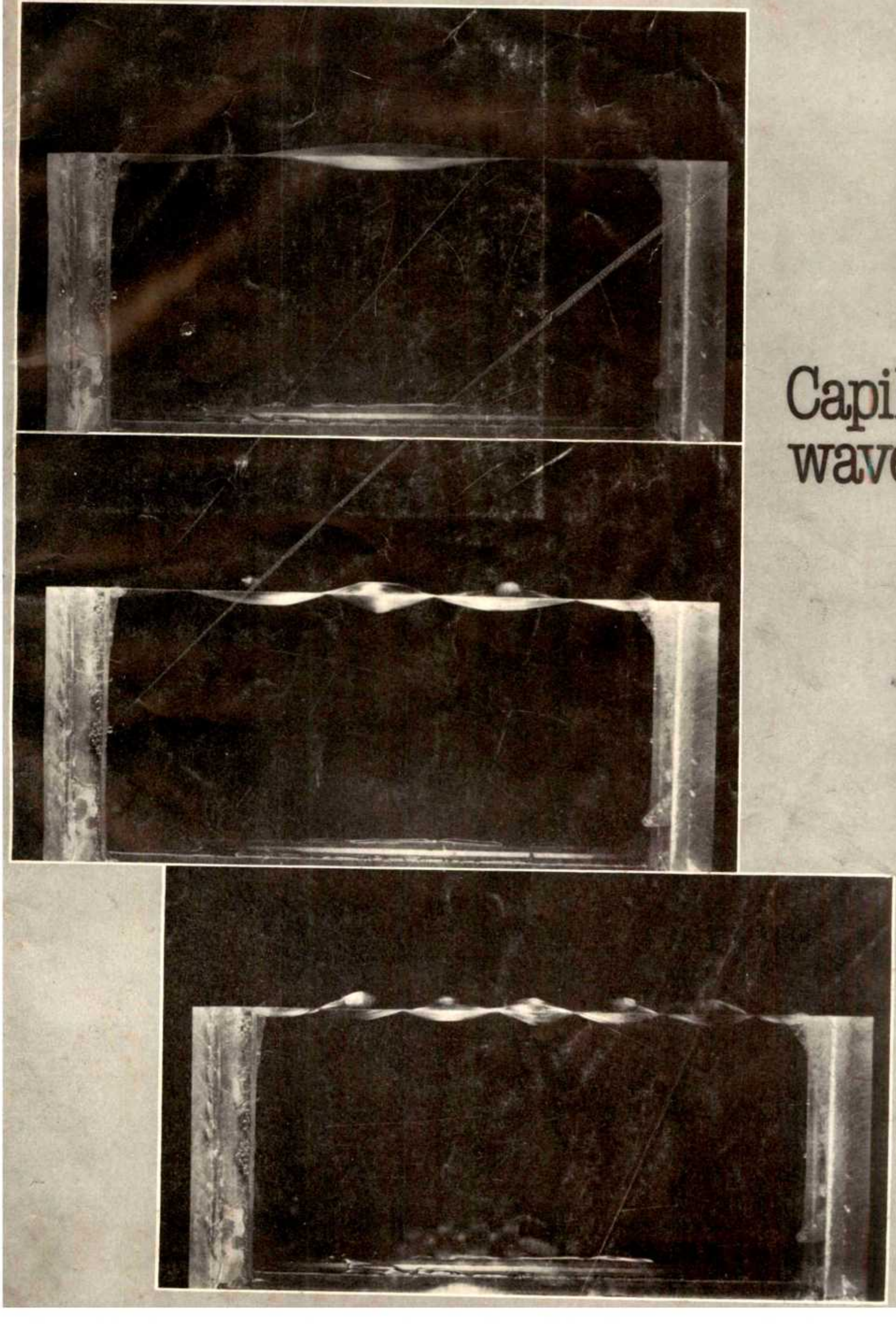

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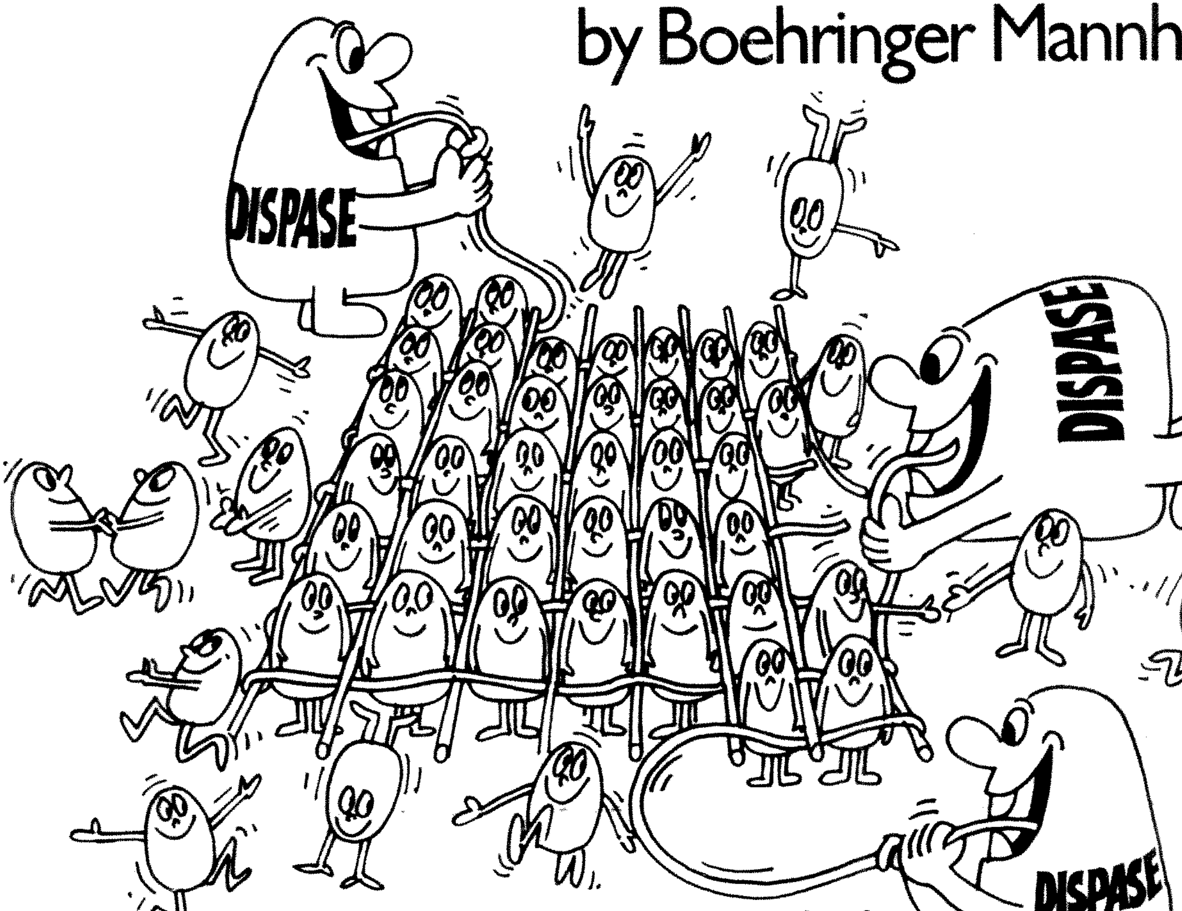

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Cover picture

The first three symmetric modes of standing capillary waves in a narrow channel. The length of the channel is 100 mm, the width (perpendicular to the plane of the page) is 15 mm. The waves travel faster than theory predicts. See page 803.

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nature

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EMBO

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Chromatin	Dr. G. Milanesi	Laboratorio di Genetica Biochimica ed Evoluzionistica Via S. Epifanio, 14 27100 Pavia ITALY	23 — 26 March Courmayeur 50
The Molecular Biology of the Gene	Professor M. Birnstiel	Institut für Molekularbiologie II Winterthurerstr. 266A 8057 Zürich SWITZERLAND	18 — 22 March Davos 50
Muscle Cell Culture in the Study of Gene Expression during Differentiation	Dr. M. Shani	Department of Cell Biology Weizmann Institute of Science Rehovot ISRAEL	October Rehovot 75
Chloroplast Membrane Polypeptides	Professor D. von Wettstein	Department of Physiology Carlsberg Laboratory Gl. Carlsbergvej 10 2500 Copenhagen Valby DENMARK	11 — 16 June Copenhagen 30
The Nerve Growth Factor and other Specific Growth Factors	Professor R. Levi-Montalcini	Laboratorio di Biologia Cellulare Via G. Romagnosi 18/A 00196 Rome ITALY	early in October Rome 40-50
Comparative Study and Recent Knowledge on Quaternary Structure and Active Sites of Oxygen Carriers and Related Proteins	Professor J. Lamy	Laboratoire de Biochimie Faculté de Médecine 2, bis Bd. Tonnellé 37032 Tours Cédex FRANCE	20 — 24 August Tours 50
Concepts of Chemical Recognition in Biology	Dr. F. Chapeville	Laboratoire de Biochimie du Développement, Institut de Recherche en Biologie Moléculaire du CNRS 2, Place Jussieu-Tour 43 75221 Paris Cédex 05 FRANCE	17 — 21 July Thiverval-Grignon 70
X-ray and Neutron Small Angle Scattering of Biological Structures	Professor H. Stuhrmann	European Molecular Biology Laboratory Notkestieg 1 2 Hamburg 52 FEDERAL REPUBLIC OF GERMANY	24 — 28 September Hamburg 40-60
Polypeptide Neurotoxins: Structure and Function	Professor M. Lazdunski	Centre de Biochimie Faculté SPCNI Université de Nice Parc Valrose 06034 Nice FRANCE	24 — 27 July Nice 50-80
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Cell Lineage, Stem Cells and Determination	Professor N. Le Douarin	Institut d'Embryologie CNRS 49bis, av. de la Belle Gabrielle 94130 Nogent-sur-Marne FRANCE	20 — 24 May Onzain 40
Molecular and Cellular Aspects of Obesity	Professor G. Ailhaud	Centre de Biochimie Faculté des Sciences Parc Valrose 06034 Nice FRANCE	27 — 29 August Nice 50-70
Erythropoieses and Differentiation in Friend Leukemia Cells	Professor G. B. Rossi	Laboratorio di Malattie Batteriche e Virali Istituto Superiore di Sanità 299 Viale Regina Elena 00161 Rome ITALY	September/November Urbino 60
Control of Replication and Partitioning of Bacterial Chromosomes and Plasmids	Professor R. Pritchard	Department of Genetics University of Leicester University Road Leicester LE1 7RH ENGLAND	September Leicester 45
H-2 Antigens: Genetic Control, Expression and Function of Normal, Tumour and Virally Infected Cells	Professor H. Festenstein	The London Hospital Medical College University of London Turner Street London E1 2AD ENGLAND	29 April — 3 May London 25

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- Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.
- 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

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(ii) first and last pages of references should be cited.

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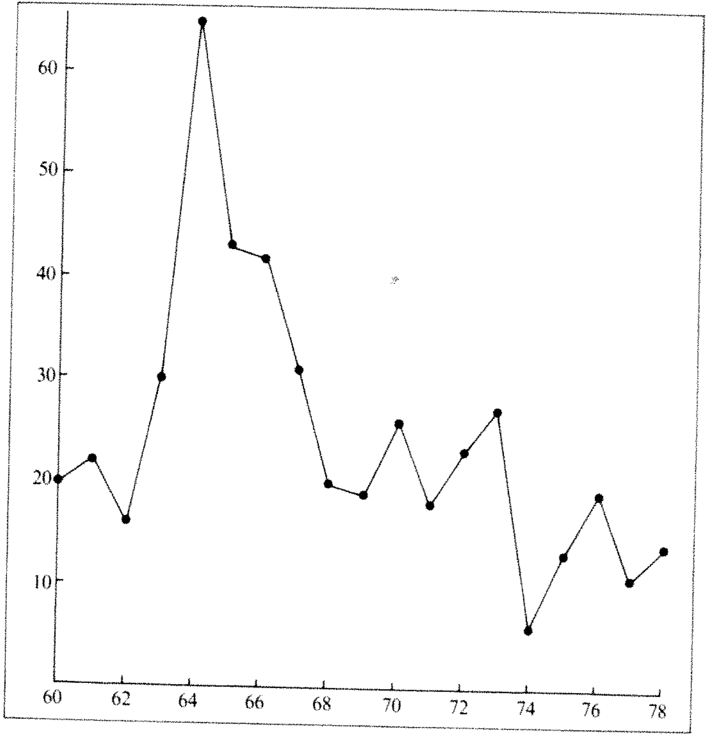
nature 21/28 December 1978

No end in sight to jobs gloom

Four years ago (Vol. 253, p. 79) we looked at the fluctuations in job opportunities in British universities since 1960. Now is a suitable time to bring the record up to date. The graph shows in a schematic way numbers of tenured posts available up until the end of 1978.

The technique used is the same as that employed in 1975. The classified advertisements in *Nature* for two weeks, in May and November, of each year were examined. Posts at or above the level of lecturer at a British university scored one. Temporary posts scored half. The figures for May and November were added together. Various objections can be raised; that not all jobs will be advertised in *Nature;* that the method of measurement, though consistent, is crude; that many universities have research-council establishments attached to them which perhaps should also be included; that there are large research-council and other establishments not attached to universities but which offer at least a semi-academic environment. Nonetheless, it is likely that the graph is a moderately good guide to the ups and downs of employment prospects for the academically inclined.



The enormous spike in the mid 1960s need not detain us long. This was the time when new universities were rapidly coming on stream and recruiting with immense enthusiasm. This cohort of new-university staff is now largely in its 30s and 40s and unlikely to retire for some time. In the late 1960s recruiting seemed to return to its old level but then in 1974 many university posts were 'frozen', owing to a major economic crisis.

What has happened since then? There has been no obvious rebound; it must be assumed that many of the frozen posts have never re-appeared. In fact the level of recruitment in the past four years looks unmistakably down on that of the early 1970s, for which the present low-level of retirements from universities must take part of the responsibility. Nor is there much comfort just over the horizon. The numbers of students choosing to do science at university is certainly not growing quickly enough to warrant major increases in staff; indeed in recent years numbers have often declined. And the demographic signs are unmistakably that young people of university age will be substantially less in number in ten years' time than they are now, so teaching opportunities are hardly likely to increase.

At present a large and growing number of post-doctoral research workers find themselves caught in the trap. Tenured posts are few and far between whilst short-term contract research merely delays the day of decision and offer no security. The recently formed Association of Researchers in Medical Sciences, intended to articulate the concern of medical researchers thus trapped, could easily have a counterpart in other branches of science.

No one simple solution will emerge to this very serious problem. The encouragement of early retirement is likely to increase, and will help, at least temporarily. Specific initiatives have helped in some particularly difficult circumstances. But in the long run the responsibility may lie as much as anywhere on those tenured university staff who take on new graduates to study for a Ph.D. In the flush of satisfaction at having found just the right person to solve the problem in hand, it is all too easy not to make discouraging noises at the same time about long-term job prospects. Research students should be told in no uncertain terms that a Ph.D. is no longer a guarantee of entry into the academic world and in some circumstances overqualifies for non-academic jobs and so could even be a liability. Any research supervisor who fails to make potential students think twice about the path on which they are entering is failing in one of his most important responsibilities. □

US extends recombinant DNA controls to private industry

A SERIES of moves was announced this week by Mr Joseph A. Califano, Jr, US Secretary of Health, Education and Welfare, designed to extend the guidelines currently covering federally-sponsored research involving the use of recombinant DNA techniques to all such research carried out in the private sector.

In particular, Mr Califano has directed the Food and Drug Administration to use its statutory authority to require that all recombinant DNA research submitted to satisfy regulatory requirements—for example in order to licence a new drug—comply with the guidelines that have been developed by the National Institutes of Health.

And as regards research in the private sector which would not be submitted to the FDA in this way, he has requested the Environmental Protection Agency to take all the action it can "to require that recombinant DNA research conducted by the private sector complies with the NIH guidelines."

Mr Califano also announced that he has approved final guidelines prepared by Dr Donald Fredrickson, director of NIH, that significantly revise the safety requirements for conducting recombinant DNA research.

As expected, the revised guidelines substantially broaden public representation on the Recombinant DNA Advisory Committee, which assists the NIH in administering the guidelines. It also increases public participation in the administration of the guidelines by local biosafety committees, as well as public access to information about recombinant DNA research.

Finally, since the revised guidelines require the director of NIH to determine that any proposed action under the guidelines "presents no significant risk to health or to the environment," Mr Califano has directed the NIH to formulate a balanced programme of risk-assessment experiments at NIH directly, or under NIH support.

"In my view, the more risk assessment experiments NIH carries out, the better we will be able to judge whether the guidelines—and actions taken under them—afford appropriate protection for health and the environment," he says in a memorandum to Dr Fredrickson and Dr Julius Richmond, Assistant Secretary for Health.

In a public statement announcing these various actions, Mr Califano says that the final version of the NIH guidelines based largely on proposed revisions published in July, relax in two major respects the guidelines that were placed in effect in 1976.

The first is that the revisions exempt altogether five categories of experiments, which the NIH has concluded present no health risk. This will exempt approximately one-third of the research now covered by guidelines.

The second relaxation is a general easing of restrictions on other permissible experiments. Almost all categories of research are assigned to physical containment and/or biological containment levels at least one step lower than in the 1976 guidelines.

"Since the likelihood of harm now appears more remote than was once anticipated, the scientific community has now concluded that this downgrading is appropriate," Mr Califano says.

A number of significant changes to the proposed revisions have been introduced to the ways in which the guidelines are administered, following a review carried out by a departmental committee chaired by Mr Peter Libassi, DHEW's general counsel.

In particular, whereas the 1976 guidelines had no specific requirements for public participation, it is now specified that 20% of the members of local institutional biosafety committees (IBCs) must represent the public, and have no connection to the institution.

It is also now required that the same proportion of the Recombinant DNA Advisory Committee be "persons knowledgeable about such matters as applicable law, standards of professional conduct and practice, public and occupational health, and environmental safety". And it is recommended that one member be a "non-doctoral" person from a laboratory staff.

To take in these extra interests, the membership of the committee is to be increased to 20, and the new members are expected to be announced by Mr Califano in the near future. Furthermore all federal agencies represented on the Federal Interagency Advisory Committee will have non-voting members on the RAC.

One result of demands for increased public participation is that all important records relevant to research carried out under the guidelines must be made public. In particular, the bulk of IBC records must be made available to the public, and problems, violations of the guidelines, illnesses and accidents must be reported to NIH.

Major actions under the guidelines—such as decisions to approve on a case-by-case basis experiments that are generally prohibited, to exempt additional categories of research from the guidelines, or to permit the insertion of genes into new types of bacteria—cannot be taken without the advice of the RAC, with public and federal agency comment on the proposals.

Califano: more risk assessment experiments

In addition, the Director of NIH cannot approve any proposed actions unless he determines "that they present no significant risk to health or the environment". This implies the need for some type of risk assessment.

In a statement accompanying the final version of the guidelines, Dr Fredrickson says that one reason for distinguishing between the hazards of different organisms on the basis of phylogenetic relatedness—a subject recently debated in Britain's Genetic Manipulation Advisory Group (GMAG)—is that the more closely related the species, the more likely that polypeptide hormones or related proteins would be pharmacologically active, particularly in the case of recombinant DNA integrating into the human genome.

"This matter lies in a crucial area of risk analysis and will undoubtedly continue to be a subject of both further debate and improved understanding in the coming months", Dr Fredrickson writes.

The final guidelines also include detailed presentation of the types of responsibility to be delegated to the local biosafety committees. Dr Fredrickson says that although this will place a burden on the human and financial resources of the institution, the responsibility is better delegated than retained at the federal level.

A new proposal will allow NIH to require prior approval of all recombinant DNA research if the institution fails to comply with the guidelines.

David Dickson

UK researchers claim rights over short-term contracts

At least two scientists believe universities and research councils are treating post-docs shabbily, writes **Joe Schwartz**

Two British scientists are turning to the courts and industrial tribunals to find redress over dismissal at the end of short-term research contracts. Dr Desmond Turner has brought a tribunal hearing against the University of Sussex; and Dr Amiram Ur is bringing another, continuing this week, against the MRC's Clinical Research Centre in Harrow.

Dr Turner, 39, was asked to leave the University of Sussex in June at the end of an MRC-funded project to isolate gastro-intestinal hormones involved in the regulation of insulin secretion. Dr Ur, 44, was also asked to leave, in his case after limited-term contracts totalling eight years' work with the MRC at Harrow.

Both have been productive scientists. Turner has published 15 papers relating to the hormonal regulation of insulin secretion over a period of 14 years on short-term contracts. Ur, a bioengineer, has numerous patents, most notably an impedance measuring device for monitoring blood coagulation and bacterial cell growth which is patented in 18 countries and is marketed in the UK under the names Biobridge and Bactbridge.

Turner and Ur have found themselves at the centre of a legal and political thicket. First, the legal status of short-term contract workers is not at all clear. Despite the fact that post-doctoral fellowships, for example, extend only for an agreed fixed term, there appear to be rights under the Employment Protection Act, 1975, to redundancy pay and claims over unfair dismissal at the end of the contract. But increasingly employers—universities and research councils—have been introducing a 'waiver clause' into the contracts which nominally eliminate those rights.

Turner says: "There is no justification for the use of special forms of contract that deprive employees of their normal rights under the law. . . . We need to bring to public notice the shabby way in which institutions are treating temporary appointments".

On the one hand it would seem sensible that the granting institutions should be free to allocate money according to their own perception of scientific merit without fear of redress. On the other, it would also seem fair that the increasing number of experienced post-doctoral and other short-term contract researchers (perhaps 10,000 in the UK) should find some security of employment. Mr P. C. Stephenson, Secretary of Science at the University of Sussex and responsible for Turner's employment, recognises the problem but feels that the Government and the Association of University Teachers (AUT) are responsible for creating a coherent national policy. "We are aware of the problem but we need to find the right place to start the argument," says Stephenson.

The legal position of contract workers became more complicated on 26 July 1977, when an Industrial Appeals Tribunal ruled in L. D. Dixon v. BBC that Dixon's contract was not fixed term during its tenure, thereby invalidating the waiver clause, but that it became fixed term at its point of expiration, thereby entitling Dixon to redundancy pay. In October of this year, an appeal court overruled the Appeals Tribunal and decreed that the waiver clause as customarily employed was valid.

Following this decision the AUT withdrew from Turner's case. However, Turner, with the aid of Brian Salter, chairman of the research subcommittee of the AUT's Sussex University branch, took his case to the tribunal hearing on 27 November, 1978. "We felt we still could make a case and we wanted the tribunal to commit itself to say that the appeal court ruling on Dixon v. BBC was relevant to research workers," says Turner. The tribunal has not yet made its decision.

Salter, who is encouraging researchers to press redundancy claims, agrees that a coherent national policy is called for, but says that the AUT is not sufficiently aware of the conditions of research workers. Salter feels that bridging loans betwen contracts, reorganisation of the nation's research into core areas located within universities and a well defined career structure alongside the normal university lecturer scales is needed. "The Research Councils will also have to accept that people have to be hired even when they get older", says Salter.

Salter and Turner both think that a closed union shop may be the only way for short term contract workers to get any control over their jobs at all. "Under conditions where there are so many out of work scientists, the jobs will go to the cheapest bidder to the detriment of us all", says Turner.

The case of Dr Amiram Ur, being heard again last week in London by an industrial tribunal after two months adjournment, also raises fundamental questions. In July 1974, the MRC introduced 'Establishment Code 335' which radically revised the career structure for non-clinical staff. Citing the greatly reduced university job opportunities as a reason, the MRC instituted a procedure under which, after four years, "awards of unlimited tenure will be made on average to 75% (taking one year with another) of the number of staff awarded limited-term appointments."

Approximately 600 people were affected by the order, which would result in 450 tenured posts but also redundancy for 150 scientists. Changes in the Establishment Code are negotiated agreements and as the negotiating body, the AUT is implicated in this ruling. But as one experienced observer noted, "This AUT negotiated tenure procedure is really a dismissal procedure and doesn't meet the minimum legal requirements of a dismissal system." Ur was one of the MRC's employees that came under the new ruling.

On 29 March, 1974, Ur was transferred from clinical to non-clinical status which brought him under the new requirements imposed later in the year. In November 1976 Ur's mandatory application for tenure was denied and under the new terms of service was given a year to find another job. Ur protested the decision citing deficiencies in MRC procedure beginning with his transfer to non-clinical status. This failing, Ur was dismissed in November 1977. The first tribunal hearing on the case was scheduled for April 1978. After an MRC request for postponement, in June 1978 the MRC offered Ur a settlement, which included back pay, reinstatement on a three-year contract, legal costs up to £4,000, return of pension rights and an elaborate and costly procedure to "investigate the grievances for and against Dr Ur".

The grievance procedure provided for a three-person committee, one member selected by Ur, one by the MRC and a chair person by mutual agreement. In case of difficulty with the latter choice, nominations were to be received from the President of the Royal Society. The committee was to have a paid secretary and to have had the ability to call and pay witnesses. However, its findings were to be confidential to the MRC and council actions based on the report were to be final and binding. Ur refused the settlement.

Meanwhile, the hearings of Ur's tribunal are expected to carry over until March. ☐

Better Med than Dead

Vera Rich reports on Israel's plan to save the Dead Sea from drying up by building a canal linking it to the Mediterranean

ENVIRONMENTAL protection is normally one of the first victims of a war economy. It would be tempting, therefore, to interpret the Knesset motion tabled last month by former Foreign Minister Yigal Allon, to avert ecological disaster by constructing a canal linking the Mediterranean and the Dead Sea, as a sign of approaching political normalisation Ironically, this is by no means the case; indeed, according to some Israeli sources, the signing of a peace treaty with Egypt would effectively block any such plans.

The idea of such a canal is not new. It was first proposed at the end of the last century by Theodore Herzl, the Zionist leader who first envisaged a future Jewish state. Since then, a number of plans have been proposed, including a grandiose scheme for a ship-canal down from Haifa, viá the Sea of Galilee, lower Jordan and the Dead Sea, down to the gulf of Aqaba. Present plans, however, and there are five under consideration by the Israeli government, with three possible routes, concentrate rather on the possibility of using such a canal as a source of hydro-electric power which could produce up to 10% of Israel's present generating capacity

The Dead Sea lies some 400m below the level of the Mediterranean. Theoretically, therefore, such a canal could descend via a number of hydroelectric cascades. Such a project is highly favoured by Israel's anti-pollution lobby, notably by Dr Anthony Pirano of the Techion, who has worked out his own "integrated" scheme, with desalination of the water at the inlet end, and use of the proposed storage reservoir at Arad as a winter spa. Other proposals are somewhat less elaborate, but all would admit that the construction of such a canal would not be at present justifiable economically, on the basis of the energy produced alone

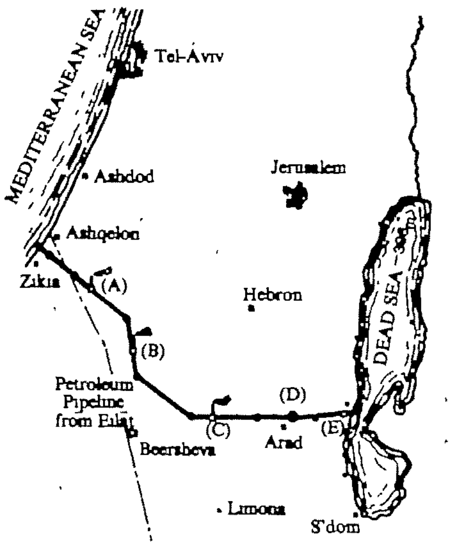
Replying in the Knesset to Mr Allon's motion, the Energy Minsiter, Yitzak Moda'i, stressed this point. Electricity, he said, can still be generated more cheaply by conventional power-stations using fossil fuels. The main purpose of the canal would not, however, be the hydroelectric scheme, although, as Mr Joseph Vardi, official spokesman for Israeli Ministry of Energy and Infrastructure told *Nature*, power production would be a spin-off which would make the canal economically feasible. Its prime purpose would be to recharge the Dead Sea

Of recent years, Israel's use of the Sea of Galilee as the water source of the whole country has led to a significant drop in the level of the lower Jordan and a consequent fall in the level of the Dead Sea. The arid climate of the Dead Sea region causes a high evaporation rate—a fact utilised by the Dead Sea potash plant at S'dom, which is based on the natural evaporation of brine from shallow pans. Already the receding shore-line has left the S'dom works some distance "inland," and it has been necessary to construct a new pumping station and canal to bring the brine to the evaporation pans.

Although the Dead Sea Works has thus solved their own immediate problem, this is no remedy for the ecological changes produced by the recession of the Sea According to Mr Allon, the situation is becoming "urgent" due to continuing progress by the Jordanians in their own water projects. The diversion of the Yarmuk river (a tributary of the lower Jordan) via the East Ghor canal for irrigation purposes is almost complete. Half the streams flowing into the Jordan from the east have already been dammed, and the damming of the remainder is proceeding at full speed. Once these Jordanian schemes are complete, said Mr Allon, the lower Jordan will dissappear completely, save as a drainage channel, the level of the Dead Sea will fall completely and the southern part of the present area of the Eea would become a salt desert This, as Mr Moda'i admitted in his reply, could cause "tremendous" ecological damage, which could not be easily repaired.

Use of the Jordan water by Israel's eastern neighbours has always been a sensitive issue. The "war over the waters" between Israel and Syria, was one of the prime causes of tension which led ultimately, to the Arab-Israeli conflict of 1956. Since it is not the loss of the lower Jordan itself, but the drying up of the Dead Sea that is seen as the ecological hazard, the construction of the proposed canal would clearly remove at least one potential cause of future conflict between Israel and Jordan.

A speedy separate peace with Egypt, however, would almost certainly force any such construction project to be deferred. Not for any political reason, but simply because Israel's resources, both human and financial are limited, indeed, overextended. Military withdrawal from the Sinai would entail the construction of new defence installations in the Negev.



Dr Pirano's proposed canal route

Furthermore, troops stationed in the Sinai have left their families at home, generally in the populous areas in the north If transferred to the Negev, however, they would probably bring their families south, with consequent development of such towns as Be'er Sheva and Dimona, as well as less fortunate settlements such as Yeruhanm which have never really become fully viable. Under such circumstances, Ben Gurion's vision of the Negev as the heartland of Israel's prosperity could well come true—but at the cost of resources which could be otherwise used for the proposed canal

According to some Israeli sources, there is considerable pressure "from outside" for a post-treaty Israel to develop a joint nuclear power capability with Egypt, the stations being situated in the Sinai, as a further incentive to peace. Such a project running into "billions of dollars" would be of considerable interest to foreign investors Israeli attitudes to nuclear power are generally cautious—particularly since the proposed coastal site for one nuclear station between Ashqelon and Ashdod (see map) was found to be earthquake-prone. Until now, however, costs have been too high for Isreal to considér any major nuclear power programme.

Should the proposed joint Israel-Egypt Sinai scheme become a reality, the proposed canal would be less important from the energy point of view, and with still more of Israel's construction workforce committed to the Sinai power-stations, its early construction would seem most unlikely.

The ecological threat to the Dead Sea would remain. It would be ironical if the peace-makers who urge Israeli-Egyptian nuclear cooperation should prove later to have laid the foundations for a second "war of the waters". ☐

Inflation forces new tactics on US environmentalists

LAST SUMMER the environmental sciences board of the US National Academy of Sciences prepared a list of topics requiring its attention. At the top of the list was the potential threat to schemes for environmental management and regulation posed by pressures on the economy, and the political measures which might arise.

The board's concern reflected a debate that has been simmering in Washington since President Carter's administration began its attempts earlier this year to take a "firm grip" on the problem of inflation. A major component of the ensuing debate has been whether environmental controls are inflationary; and if so, what should be done about them.

Concern among environmentalists erupted briefly at the beginning of the autumn when Mr Robert Strauss, then the administration's chief inflation fighter, let slip his view that environmental regulation was one of the first areas in which cut-backs could be made.

Since then the rhetoric has been softened. Administration economists now talk about the need to demonstrate the cost-effectiveness of proposed regulations; and both regulators and environmentalists have won a number of important battles. But they are now on the defensive.

On balance, 1978 has not been a bad year for environmentalist causes. Though backing away from some commitments, President Carter has put up a strong fight against many potentially damaging water projects opposed by groups such as the Sierra Club, and recently signed into law the preservation of large areas of Alaska.

Furthermore last week the Tennessee Valley Authority announced after years of opposition, that it was prepared to invest over $1 billion to clean up air pollution at ten of its power plants, a settlement described by the Environmental Protection Agency as "the largest ever made with a major source of air pollution".

However just as four years ago environmental regulations were being blamed for the problems of increasing unemployment, so the same is now being widely said about their contribution to inflation; industry claims, for example, that meeting new controls means taking away money from more productive areas of investment.

In reply, environmentalists claim not only that such regulations make a very small contribution to the increase in the consumer price index—estimated at less than 0.5%—but that if factors such as reduced health care costs are taken into account, the benefits of pollution control measures outweigh the costs.

"Most important, I believe that the public wants those benefits and is prepared to pay for them," says Douglas M. Costle, administrator of EPA. A recent survey conducted by Resources for the Future found that 62% of those questioned were prepared to pay higher prices to reduce pollution.

But to answer the charges being laid against the regulators, convictions previously expressed in qualitative terms are now having to be quantified. "We need to focus more on the adequacy of data, their interpretation, the need for risk-benefit analysis from a systems view," says Dr Gilbert S. Omenn, assistant director of the President's Office of Science and Technology Policy.

This need is reflected in the shifting strategies of environmentalist groups. Initially reluctant to place quantifiable values on that which they are trying to defend, this is now becoming necessary to respond to proposed "anti-inflation" measures. The Natural Resources Defense Council, for example, one of the strongest such groups, is setting up a working group on the economics of the environment, and others are moving in this direction.

> **'We are going to insist on better data on the cost-effectiveness of environmental control measures. I do not see this as necessarily bad, unless the pendulum goes too far'**
> —Prof John Cantlon

A major problem, however, is that whereas the likely costs of any new regulation are relatively easy to predict, the same is not true for the other side of the equation. "There is no question that trying to get at the benefits side of the picture is a very much tougher job of analysis and study," says Professor John E. Cantlon, professor of botany at Michigan State University, and chairman of the NAS's environmental sciences board.

"However it is something that needs to be done. The scientific community should not stand around wringing its hands, but should start getting research groups interested in the problem," he says.

"The climate in government and in industry broadly—and I would even say in society—is that we are going to insist on better data on the cost-effectiveness of environmental control measures. I do not see this as necessarily bad, unless the pendulum goes so far as to call into question genuine concerns about internalising the social costs of production."

Others are concerned at this happening. Speaking at a meeting last week organised by a group called Environmentalists for Full Employment, Ms Peggy Seminario, an industrial hygienist with the American Federation of Labour and Congress of Industrial Organisations (AFL-CIO), claimed that many companies were using inflation as an excuse to resist further regulation, often using suspect data to support their arguments.

"At the heart of the debates on cost estimates, there are not many numbers, and the numbers are not that good. In 1974 environmental impact statements claimed the cost of meeting proposed vinyl chloride standards would be between $65 million and $90 million; recently, after the standards were introduced, it has been estimated that the costs to industry of meeting the regulations was less than $1/2 million."

But the increasing incursion of economics into the environmental debate is also raising difficult issues within the environmentalist movement. Some have argued, for example, that the price of energy should be allowed to rise so that solar energy, already environmentally preferable to more conventional sources, can become economically competitive as well.

Others dispute this strategy, claiming that any increase in energy prices has an unfair impact on the poor. "The net result of present policies is to force people at the low end of the income distribution to lose food to pay for solar energy," Dr Gar Alperovitz, co-director of the Exploratory Project for Economic Alternatives, told the EFFE meeting.

"It is time for the environmental movement to take responsibility for what it is saying. We must reconsider the relation between strategies on prices and the desirable goals of conservation; for example there are other ways of getting to solar energy than by supporting higher prices."

Whichever way the various debates go, they seem destined, in Washington at least, to be increasingly fought on a terrain of economic argument which few can afford to ignore. As one Congressional aide put it last week: "Economists now seem to be playing the same role in Washington that scientists used to play in the 1960s."

David Dickson

news in brief

● **Death halts Greenpeace action over nuclear waste:** The first ship to carry nuclear waste from Japan to the reprocessing plant at Cap de la Hague near Cherbourg in France, has had to alter course for Barrow-in-Furness in England after one of its senior technical experts, Mr Robin Dale Hartley, died. Preliminary reports suggests that he died of a heart attack while in his bunk. Mr Hartley was an electrical engineer responsible for maintaining the cooling systems of the 26 containers of fuel rods of uranium oxide aboard the Pacific Fisher. British Nuclear Fuels Ltd, which had chartered the vessel, said that it expected that an inquest into the death might be necessary.

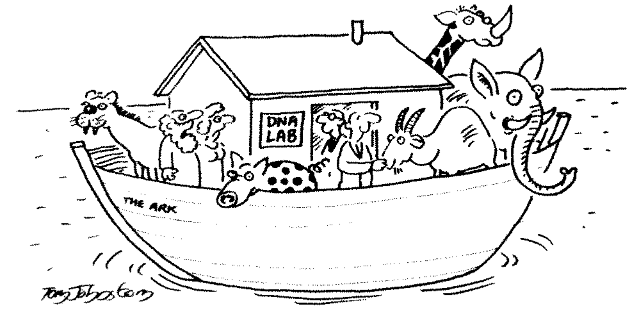
The consignment of nuclear waste is to be reprocessed at Windscale in Cumbria and at Cap de la Hague, under the terms of a contract between Britain, France and Japan by which each of the two European countries is to reprocess 1,600 tons of Japanese uranium oxide over a nine-year period from 1982. Although Britain has been taking nuclear waste from Japan since 1969, this is the first consignment to go to France.

The Greenpeace conservation group had planned to harass the Pacific Fisher as it entered Cherbourg, as part of a demonstration against shipping nuclear waste to France which involved 18 different French anti-nuclear and other political organisations. They were going to use their converted trawler the Rainbow Warrior which successfully frustrated the seal cull in the Orkneys last October.

Mr David McTaggart, European director of Greenpeace, said they were planning to run inflatable dinghies in front of the Pacific Fisher. Even though the ship is not going straight to Cherbourg, Mr Peter Wilkinson, Greenpeace director in charge of the anti-nuclear campaign, said they were still going ahead with a demonstration with the French organisations, but they were not going to demonstrate at Barrow-in-Furness, in deference to the dead man's family.

● **Museum can show Darwinian evolution:** The Smithsonian Institution's National Museum of Natural History is not required to depict the Biblical version of the creation of the universe as part of a scientific exhibition on evolution, a court in Washington ruled last week. Two religious groups claimed that a $½ million exhibition, which opens next May, violated the Government's role of religious neutrality by presenting Darwinian evolution as "the only credible theory of the origin of life". They asked the court either to ban the exhibition, or to require the museum to spend a comparable amount of money illustrating the Biblical account of the origins of man.

In rejecting both claims, US District Court Judge Barrington D. Parker said that the primary effect of the evolution exhibits was not to advance a religious theory, or to inhibit plaintiffs in their religious beliefs. "Even accepting their argument that evolution is hostile to their beliefs as to creation, this impact is at most incidental to the primary effect of presenting a body of scientific knowledge."



"Maybe it wasn't such a good idea to take along the two scientists!"

● **Thumbs up for re-usable satellite:** A favourable report on Britain's involvement in the MRS re-usable satellite project is now being prepared by a Science Research Council team and is to be presented to the council's astronomy, space and radio board in the new year. The Multi-discipline Refurbishable Satellite project, which could allow different instrument packages to be replaced while a spacecraft is in orbit, makes use of the Multi-Mission Satellite (MMS) being studied by the Americans. If it is given the go-ahead by NASA, this would lead to the setting-up of a joint UK-US feasibility study that could see the development of the revolutionary new satellite at a cost of several tens of millions of pounds.

Recently a team at the SRC's Appleton Laboratory, in collaboration with British Aerospace engineers at Stevenage, has been considering the most testing shapes and types of various scientific instrument packages that would have to be fitted to the craft. And it is now known that no serious drawbacks have been found to the various tricky configurations considered and a favourable report is to be presented to an informal meeting of the ASR board on 10 January. A more detailed report is likely to be presented to a future formal meeting of the board.

● **First evidence of gravity waves:** A team of astronomers at the University of Massachusetts at Amherst claims to have discovered evidence of gravity waves, whose existence was first predicted in Einstein's general theory of relativity 60 years ago, but has yet to be demonstrated. Using the 1,000-foot diameter radio telecope at Arecibo, Puerto Rico, Dr Joseph Taylor, Professor of Astronomy at Massachusetts, Dr Peter McCulloch, on temporary leave from the University of Tasmania, and Lee Fowler, a graduate student, monitored the radio emissions from a pulsar, discovered in 1974, which is orbiting another massive object. According to the general theory of relativity, the orbiting pulsar should radiate gravitational energy which would cause the two bodies to move closer together and thus increase the speed of rotation, which is currently a cycle every eight hours. Relativity predicts that the period of rotation should decrease by one-tenth of a millisecond each year. The astronomers found that the pulsar has slowed down by four-tenths of a millisecond, ± one tenth, in the four years since its discovery.

The results were presented at the symposium on relativistic astrophysics at the Max Planck Gesellschaft Astrophysik in Munich this week. The research workers say that they have been able to rule out other factors, such as tidal movements on the two bodies, which might have caused the changes. Calculations of the periodicity of the pulsar, which circulates its companion at 0.1% the velocity of light, have been complicated by the fact that its orbit processes at a rate of 4 degrees a year.

One physicist pointed out last week that a rotating pulsar was an almost perfect system by which Einstein's theory could be tested, since his theoretical framework was based on the concept of observers with clocks—in this case the rotating neutron star—falling freely through space.

● **Correction:** In the article "Czech Chartists claim two died in nuclear accident" (*Nature*, 276, 7 December, 1978, p.551), the fifth paragraph should commence, "A year and six weeks later, however, disaster struck again (according to the Chartists)".

Science in China: is 'the bumpy road' better?

By Stephen Risch, Michael Hansen, Judith Weinstein, Theodore Goldfarb and Carol Cina

China is bent on a great leap forward in science and technology—but it should not be at the expense of its peasants or workers

Joseph Needham's articles on science in the People's Republic of China (31 August, 1978 page 832) are for the most part informative discussions of recent changes in the organisation and process of Chinese science. However, Dr Needham's report does not accurately reflect important aspects of the political-social history of the past few years in China, and as a result draws a somewhat unbalanced picture of the evolving role of science. This is largely due to Dr Needham's uncritical acceptance of the position of the current leaders with regard to the role attributed to the "Gang of Four" (G_4), especially with respect to their influences on science and technology.

A delegation of 12 members of Science for the People[1] visited China from 4 June to 2 July at the invitation of the Scientific and Technical Association of the PRC to study scientific and technical decision-making in agriculture and food production. We travelled from Hainan Island in the south to as far north as Peking, visiting universities, research institutes, People's Communes, and factories and interviewing researchers, peasants, workers, and local and national officials. We saw much evidence of the Chinese leadership's decision to place great emphasis on science and technology to accelerate modernisation. We would like to analyse some of the important points made by Needham and present a somewhat different view of the way science is developing in China.

Needham essentially repeats the current official line in China that the G_4 caused the economy to stagnate, that their ideas were heretical to all Maoist thought, and that they nearly brought science and technology to a standstill. For instance, he agrees with the assertion that the G_4 "brought the economy to the brink of economic collapse". This simply was not so. Between 1965, the last year before the Cultural Revolution, and the most recent years for which we have estimates, production of electricity increased 157% (1974), production of steel 162% (1974), coal 76% (1974), oil 619% (1975)[2], and machinery 350% (1975)[3]. Overall industrial production was 7% higher during the first half of 1976 than in the same period of 1975[4].

Needham asserts that "the cutting off of funds for all higher institutions was, under the G_4, a matter of course", and "necessarily in alliance with this was a general despising of science and scientists". We obtained quite a different picture. Needham says the G_4 exercised a dominant influence in China during the years 1974–6. We received detailed accounts of a number of large-scale research projects that were underway then at Chungshan University and the Institute of Entomology in Canton, the Institute of Zoology in Peking, and the Shanghai Academy of Agricultural Sciences, including some that were initiated during that period.

One of the institutes that Needham singles out as having suffered most from the G_4 was the Natural Tropical Products Research Institute on Hainan Island. When we visited the Institute we found that most of the research was stopped in 1965 for eight years, that is, before the Cultural Revolution had even begun. Students and staff returned to the institute in 1973, and vigorous new research programmes started in 1975 (during the peak of the G's influence).

This example serves to illustrate a general observation we made during our visit. The Cultural Revolution clearly had a profound influence on the organisation and practice of science in China, and in many cases the level of scientific activity at research institutes and universities was slowed up or interrupted. Most researchers spent some time living in the countryside with the peasants and they devoted a considerable amount of time at their research institutes to political discussion. These policies were not instituted by the G_4 in order to punish scientists. Rather, they were principled policy decisions made by the Chinese leadership to discourage the development of a scientific elite divorced from the needs of the people and to reverse the progress already made toward that end. (This does not mean that at the local level these policies were not occasionally used as a method of punishing particular individuals; we were told that this happened.)

The redirection of science and technology was initiated at the very onset of the Cultural Revolution in 1966. In certain respects, such as the demystification of science, the decentralisation of scientific decision-making and emphasis on the practical rather than the theoretical, the new policies were a continuation and intensification of those introduced in 1958 during the "Great Leap Forward".

What is more important than assigning blame (or acclaim) to individuals for a certain science policy is to analyse what the effects really have been and will be in the future. We learned that there is still a significant difference of opinion on the costs and benefits of the Cultural Revolution.

Many scientists we talked to thought that their experience of working in the countryside had been very valuable in developing a consciousness about the needs of the peasants, whom they are supposed to be serving. They developed ideas for their investigations by discussing their work with peasants, with whom they established continuing and mutually productive intellectual exchanges. For example, Li Li-ying, head of the Institute of Entomology in the outskirts of Canton, told us how the Laboratory of Biological Control gets most of its research ideas from nearby communes. Research begun there in 1974 is centred on ways to increase the natural population density of a predator which feeds on red mites, a common citrus pest. Tang Yuan, a weed control specialist at the Shanghai Academy of Agricultural Sciences told us that before the Cultural Revolution his research work, based on techniques developed in foreign countries, was rejected by the local peasants as unsuited to the conditions in Chinese paddy rice fields. When he began to work alongside the peasants and respect their knowledge about the problems that need to be solved he discovered a suitable herbicide which is now widely used in the Shanghai area. He now believes that intellectuals should integrate themselves with workers and peasants.

We also heard complaints however, from some scientists and technicians concerning their experiences during the

Manual work for students and scientists was policy not punishment

Cultural Revolution. They complained of serious interruptions to their research work by long periods of manual labour. In some cases no use was made of the training and skills of these researchers. We could not find out why the treatment of these individuals was so different from others.

Two universal complaints we heard were about the deemphasis of theory and a nearly total disregard for foreign scientific developments. While we are pleased with the reversal of these policies by the present Chinese leadership, our discussions raised some serious questions about potential problems that may result from the present drive to develop rapidly a large scientific and technical workforce as the key to modernisation by the year 2000.

First, we were told that a system of "key" schools (including secondary schools, universities, and agro-technical colleges) has been re-instituted. Set up at the national, provincial, and county levels, these schools are endowed with better facilities and faculty than most schools and their function is to channel the "brightest" students into the best schools in the hopes of rapidly producing a large work force of better scientists and technicians.

In addition, a system of nationally standardised exams was reinstituted this year. We were told that the results of these exams will be the most important criteria in deciding which students go to universities and which are sent to key schools. Further, the old requirement of students spending one or two years in manual labour before pursuing advanced studies has been dropped. Vice chairman Teng Hsiao-ping said in his 18 March speech, "Those who labour, whether by hand or brain, are all working people in a socialist society". Some scientists we spoke to agree with this idea. Others, however, suggested that all intellectuals benefit from manual labour and contact with the masses. We also learned that the government has decided to give special perquisites such as higher wages and better living conditions to scientists.

Associated with these policy changes is the danger of the development of a scientific elite that will become separated from the masses and do work unconnected to the needs of the peasants and factory workers. During the years of the Cultural Revolution there was much concern about the possibility of such an elite developing in China, as had happened in the Soviet Union. The fact that China was a society in transition to socialism meant that social classes still existed. Before the Cultural Revolution most scientists and technicians came from the upper classes. Thus, at that time, the leadership saw the division of mental and manual labour as a form of class antagonism with the upper classes performing primarily mental labour and the peasants and workers primarily manual labour. Consequently, they emphasised the need for scientists and intellectuals to engage in political study and perform manual labour alongside the masses so as to better understand their living conditions and be reminded of who their knowledge and science should be benefiting. Virtually everywhere we went this summer we were told how important it was that scientists develop a proletarian outlook so that their work would serve the needs of the people, but some of the new policies seem to conflict with this goal.

Throughout our trip, however, we did see evidence of integrating science and technology with agricultural and factory work. An excellent example of this is China's Four-Level Agro-science Research Network. This network involves agricultural workers in research and decision-making in agriculture-related science work. It consists of a county level research institute and a team of agricultural technicians and trained peasants for each county, commune, brigade, and production team[5]. The teams of technicians are engaged in agricultural research, agro-science, education, and popularisation of both the results and the methodology of scientific farming. We studied the network in detail in Wu county, Kiangsu province. Eighty per cent of the counties in China have been similarly organised since the networks were initiated in 1974.

We would like to emphasise that we did see considerable evidence that science will continue to serve the people of China. Yet, the political turmoil of the Cultural Revolution has subsided over the last several years. In its place, there has been an increased emphasis on stability and economic growth. Both Dr Needham and the editor of *Nature* apparently view this as a positive development: "So what do the Chinese scientists need most at present? They certainly need a period of many years of tranquility, away from the limelight of political attention . . . Science cannot survive intact given such a bumpy road". We are not so sure.

If one measures progress in science only in numbers of publications, research projects and highly trained scientists, perhaps they are right. At one time at least, however, the Chinese decided that this was not to be the measuring stick for their science. Rather they said that the best science comes from researchers who are fully integrated with other workers in the society and who are committed to serving the needs of the broad masses of the population. At times this may slow down the rate of scientific growth, as measured by Western standards. During the difficult transition to a socialist society, it may be that the "bumpy road" is the only way to achieve this alternative vision of science. Developments in China during the next few decades may help answer this question. □

Notes

[1] Please address comments and questions to Science for the People, China Delegation, 897 Main Street, Cambridge, MA 02139. (The views expressed in this article do not necessarily represent the views of all members of the delegation.)

[2] "The Chinese Economy in 1976", pp 362–4, 382, *China Quarterly*, June, 1977.

[3] USCIA "People's Republic of China: Handbook of Economic Indicators", p 1, August 1976.

[4] *China Quarterly*, note 9, June 1977.

[5] A typical county has approximately 1 million people; a commune, 30,000 people; a brigade, 1,500 people; a production team, 200 people.

Frelimo concentrates on the practical side of science

Pamela Smith and David Wield recently spent two years in Maputo at the Ministry of Health and the University Eduardo Mondlane. Here they assess the state of science in Mozambique

MOZAMBIQUE, independent since June 1975, almost ten years later than most of her northern neighbours, spent this last decade of Portuguese colonialism fighting an armed liberation struggle. The necessity to gain independence through fighting Portugal, herself a backward country, had some positive aspects. By independence, for example, Frelimo policies were already clearly formulated and tried out in the 'liberated zones' of the north, which covered about one-third of the country's total surface area. These policies had obvious implications for higher education and research and as such for science.

Higher education was to exist therefore, not to train a small elite in advanced techniques, but to "permit access to scientific and technical knowledge by the peasants and workers, for them to be able to assume economic power and increase production for their own benefit." This statement was issued as part of a policy document emanating from a National Seminar on Technical Education, held in December 1975. It is further reinforced by the report of the Central Committee of Frelimo to the third congress in February 1977, stating that "In education, it is indispensible that we continue to promote a constant increase in technical and scientific knowledge among the labouring classes and guarantee their access to the higher levels of education." This position originates from early on in the history of Frelimo, when in 1968, the only Frelimo secondary school, based in Tanzania, was closed after students refused to go into the 'liberated zones' for their holiday activities, demanding instead higher education opportunities in foreign countries, far away from the realities of the armed struggle.

Policy in independent Mozambique continues to be formulated by Frelimo. It is then analysed at the planning ministry responsible, as its name suggests, for coordination and overall planning, and by commissions, including members of the various ministries, such as education, health, agriculture, industry, concerned with particular areas of study. Before any plan is made, discussion takes place at all levels, including the individual institutions, such as factories or schools where representative committees will have been elected. In a factory, for example, the workers elect a workers' council, which has responsibility for suggesting measures to improve safety at work, or to increase productivity and quality of products. They may also make suggestions for the type of training courses required for different grades of personnel from inservice to undergraduate programmes. Proposals are then made on the basis of discussion reports received by the Planning Ministry and analysed once more at ministry level. Any criticisms or further suggestions are then directed back to the planning ministry. This two way process between the centre and the periphery is very important to keep policies based on everyday realities.

Mozambique is now coming to the end of the second year of her three year plan, outlined during the third party congress in February 1977. The plan's overall objective is to raise the economy to pre-independence 1973-levels, and to develop by 1980 the basic structure for the next period of national reconstruction. The plan, as it relates to science, is taking place on two levels : first in education, where as many people as possible are to be provided with the technical and scientific skills necessary to the development of the country; and second through research mainly in certain priority areas, such as geology and crop production. Unlike many other third world countries, Mozambique does not possess a national science policy research council. Science policy is seen as an integral part of an overall plan, and as such, statements related to research can be found in the economic and social directives in the third party congress documents.

How can the two level objective be put into practice, given the colonial education system which gave only 5-10% of Mozambique's population the skills of reading and writing? Or which set up a few luxury secondary schools and a university in Maputo (formerly Lourenço Marques) for the Portuguese settlers and a handful of African and mixed race 'Assimilados'? Research done at the university and government research institutes, was for the most part irrelevant to Mozambique's needs and only questionably useful to the colonial power herself. Portugal, though poor and backward as a nation, under the Salazar and Caetano regimes, was bent on the pursuit of a high level of research to gain international prestige. Even in the Mozambican agricultural sector, with large exports, research was very restricted in comparison with neighbouring colonies like Tanganyika, which at least had competent research programmes and well staffed institutes for the major export crops such as sisal, coffee and cotton.

Camera Press

The president of Mozambique, Samora Machel, said when visiting the university in May 1976, "It is not by chance that we find that our university engineering graduates working in generating stations cannot begin to repair a generator, and medical professors who have difficulties diagnosing simple illnesses of the local population". Not only was the top scientific education that Mozambique had to offer at independence virtually useless for national reconstruction, but the schools' system was also highly theoretical and restricted to a minority, leaving an illiteracy rate of over 90%. Only those in the elite 'liceu' secondary schools could enter the University. Those in the industrial and commercial secondary schools, had to be satisfied with technician training in lower level technical institutes.

It is for this reason that in the early years of independence priority has been given to building the educational base, particularly in the rural areas. Crash literacy programmes have begun using not only school and university students but also those who are themselves barely literate, to teach those who are not. The primary education system, which takes four years to complete, has trebled since 1974 and now about 1.2 million pupils are registered.

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New schools are often set up through local initiative, and in the absence of buildings pupils can be seen learning beneath the trees of many Mozambican villages.

At independence, the secondary schools shrank rapidly as the Portuguese settlers left, but there are now over twice as many schools and pupils as in 1974. In the university, founded in 1962, the student population of over 2,000 in 1973 diminished to one-quarter of this number in the early independence period. University teachers and technicians also left in great numbers, being part of the settler and 'assimilado' population, with the result that faculties and research institutes were denuded of qualified personnel.

Further data on scientific training, manpower and budgeting is difficult to obtain. Colonial statistics were either incorrect—for example, the last population census in 1970 underestimated the population by over 20%—or non-existent. Mozambique is one of the few countries in the world with no government estimate of gross national product. However a national statistical centre is in formation this year, and a national accounting system will be organised by 1980. More comprehensive educational tatistics are being collected this year but are not yet available.

Nevertheless it is possible to estimate that there are now 200-400 Mozambican graduate-level scientific workers, mostly involved in agriculture, health, education and engineering. The majority of these are under 30 years old, and were trained in the pre-independence period. They therefore had to make a positive decision to remain in Mozambique, helping to keep services running amid the initial confusion that ensued as many of their colleagues left for Portugal. There are a few Mozambicans with postgraduate qualification from Portugal, South Africa, England, France and USA. As the policy of the government was to keep the university and research institutes going, these Mozambican scientists find themselves working alongside a considerable number of foreign scientists, some of whom remained from the pre-independence period to help national reconstruction, as well as those recruited since 1975 from socialist and progressive countries and groups.

The university and research institutes work under the general orientation of the ministry of education, placing their 'human and material resources . . . at the service of the social and economic priority sectors", as defined during the third congress. A scientific worker may therefore find him/herself engaged in curriculum planning, teaching university students and technicians, organising fieldwork and research projects and serving on interdisciplinary commissions at ministry level. Within the university, some changes have been effected. Courses have been reduced from five or six years' duration to three. In 1975, for example, the final year agronomy students decided that they did not wish to graduate until they knew something of the agricultural reality of the country. Instead of graduating, they volunteered to work in the countryside for a period. Other courses have since become more practically orientated. Last year, for the first time, chemical engineering students learned control engineering and smelting practice, in local factories as well as in the classroom. Previously, they would have conducted routine experiments solely within the laboratory and studied theoretical mathematics and physics. Hence for example, complex atomic theory has now been withdrawn from the curriculum, permitting a greater emphasis on more relevant theoretical and practical work.

Literacy is the educational priority, particularly in rural areas

In addition to existing courses, university teachers have been asked to set up new ones for lower level students. Some of the teachers were unhappy, but the majority perceived a way of changing elitist attitudes in the university. Pre-university courses for workers and crash teacher training programmes have begun. Only about 20% of the students are fulltime, the others provide technical backup in the ministries, schools, factories and other government institutions. The university's function was described by the president as having "to leave its doors and go to the factories and villages, putting its techniques at the service of national reconstruction. There it will learn new techniques from practical life. This will stimulate the study of scientific realities, not in an abstract way, but in a creative manner, linking its knowledge to the material wellbeing of the population."

New research priorities and methods of working have thus emerged. As already indicated, there is a close link between the university and the various ministries, particularly in the field of scientific research. Scientists are working in the ministry of health and the faculty of medicine; others are based in the agronomical and veterinary faculties, the ministry of agriculture and two research institutes in Maputo or small up-country laboratories.

One specific example of the type of research project that is being undertaken in Mozambique, is in the field of traditional medicine. Two hundred plants have already been collected which traditional healers claim have medicinal properties. Library research is currently being undertaken by a commission in the ministry of health, to see what is known about them. It is already clear that most traditional treatments for diarrhoea contain tannin. Within a short period, some plants which the literature search suggests are safe, will be tried out, not by extracting the active ingredients and turning them into modern pharmaceuticals, but by encouraging rural health workers to use them in the traditional manner, such as in tea form.

In the university, research has been geared to seeing how technology can be adapted and used according to priority needs. One final year student of chemical engineering carried out an important project on charcoal production, charcoal being the fuel used by the majority of Maputo's 800,000 population. Deforestation of the surrounding areas of the city seems to be one of the problems resulting from present production methods. Her work was to look at these methods not only to see how to improve their efficiency, but also to check on possible extraction and use of methanol and ascetic acid from wood distillation, since Mozambique does not produce either of these chemicals. She was also investigating the effect of centralising production on family level charcoal producers, dependent on this for their livelihood. This project has since been integrated into the planning of a larger project, based on the ministry of agriculture's forestry department. Also within the university, an appropriate technology unit has been set up, working on problems such as methods of grain storage, food preservation and water purification, especially relevant to the rural areas. In the ministry of agriculture, soil studies have begun as have studies of local foodstuffs and their usefulness for poultry rearing.

Sometimes, useful research results come unexpectedly. A recent study on the extent of migrant labour from Mozambique showed that returning labourers have technical skills which are in short supply. Another example is the national vaccination campaign, which is almost complete, having begun in 1976. Mobilisation has been extremely good and WHO estimate that already 92-96% of the population has been vaccinated against smallpox, tuberculosis, DPT (Diphtheria, pertussis, tetanus) or measles, depending on age. The 1970 census figure of 8.3 million has already been exceeded and estimates expect final totals to be in the region of 11 million. The vaccination campaign also gives demographic information on the age distribution and density of the population. In the past four years, Mozambique has been able to restructure its educational system placing the main emphasis on basic skills. Its higher scientific manpower, small in number, has been actively involved in this restructuring in teaching, planning as well as research. Teaching has become more practical, planning has had to take into account national priorities and research has thus been research with a small 'r', requiring only restricted laboratory and library facilities and using students as well as teachers.

This year, one of the priorities of the university and government research institutes has been to plan for research in the medium term that is for the next planning period beginning 1980, not as until now, on a short term basis. Stricter planning is required and the lessons learnt from the last few years, are being analysed in detail so that improvements can be made ensuring that research tasks are put more, not less, into line with national priorities.

The Mozambican government continues to recruit scientists able to work in various fields. Those who feel that they may have relevant skills and could be useful in the Mozambican context, should write to: — The Mozambique Information Centre, 34, Percy St., London W1P 9FG, Telephone No. 01-636 7108 who coordinate recruitment in Britain and would be happy to receive enquiries. ☐

'By nature not nurture' they sang . . .

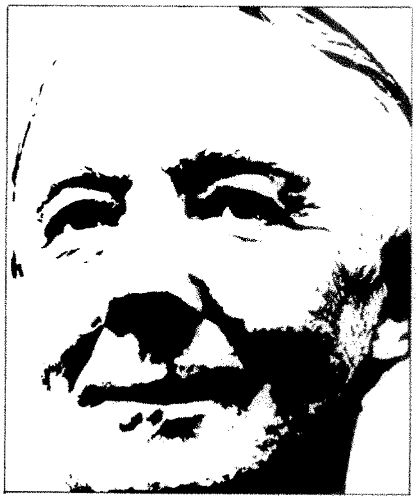
AT THE recent International Conference on the Unity of the Sciences in Boston in the United States of America, the life sciences committee, of which I was chairman, had a useful, calm and objective discussion on genetics and society, when the importance of inheritance and environment in man was considered. One of those present was Professor Arthur Jensen from the Institute of Human Learning of the University of California. At the end of the meeting he said that he had been very favourably impressed by the behaviour of the discussants and of the audience; all shades of opinion had been expressed without fear of interruption. He compared this experience to that obtained before audiences in universities in America and Europe, where certain left-wing groups have disrupted the meetings, saying that these topics should not be investigated or publicly debated.

It is interesting to note the changes which have occurred in the attitudes of the politically motivated in this field. In the 1920s there were many Socialist Sunday Schools in Glasgow, Scotland, where young people were nourished in what was then considered to be pure Marxist doctrine. Techniques of instruction familiar to the audience, many of whom had previously attended Church Sunday schools, were used. They listened to "sermons" and sang secular "hymns". One favourite hymn included the lines:

"By nature not nurture we'll rise
to the skies
The means of production we'll
nationalise."

Although nationalisation may still remain within socialist political programmes, views on nature and nurture appear to have changed. Fifty years ago those running the Sunday schools took the view that those of their pupils with innate, inherited ability would triumph over the effects of their unfavourable environment. In fact they believed that the effects of heredity could be even more important than those of the environment, if the individual then received the appropriate type of education. Professor Jensen and others have, in recent years, been vilified for suggesting that this may still apply.

There has also been a change in attitude to the use of Intelligence Quotients (IQs). Left wing opinion



KENNETH MELLANBY

condemns these as instruments in promoting racism. However, at one time these were considered the fairest method by which bright children from poor educational backgrounds, who found conventional examinations difficult, might be admitted to grammar schools. The intention was not to show that the underprivileged were stupid, but to give them a better chance to develop their potentialities.

I found IQ tests to have some value when selecting students to admit to the first university courses we started at Ibadan in Nigeria in 1948. Our infant university could only admit a very small proportion of those who applied. Nigerian schools at that time were very variable, ranging from the government colleges which compared with grammar schools in Britain, to those run privately with few trained staff. We set what were then considered conventional written papers in arts and science subjects, and I also gave each candidate an IQ test. Most entrants were selected on the results of the written papers, and none whose marks where above an arbitrary minimum was excluded, but I also admitted a few who did badly in written work but who had high IQ scores.

I am glad to say that members of this latter group almost all did well in their later university courses. In fact I found that, for all students, their results in their degree examinations correlated better with their IQ figures than with their marks in the entrance examination. Incidentally the IQ scores of our successful Nigerian students were in the same range as those found with white undergraduates I tested in Britain, so these findings should not be offensive to those who, for political reasons, oppose this type of investigation. ☐

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correspondence

Biochemical nomenclature

Sir,—Every biochemist knows the frustration of being told to use a new name for a substance when he was perfectly happy with an old one. It can, however, be just as frustrating when different groups of workers use different nomenclature systems. Widely agreed recommendations may, at the very least, prevent a name from implying an incorrect structure; they may even indicate correct ones.

Slavish adherence to fully systematic nomenclature gives unusably long names; at the other extreme laboratory jargon is understood only by the initiated. There is therefore a need for specialised nomenclatures in many fields to compromise between the needs of the workers in the field and more general readers.

A difficulty in the past has been that names may be established before it is realised that they are misleading or ambiguous to those in related fields. A glaring example of this is 'diphosphate'. This was long used to mean two phosphate groups, and also, as in ADP, a single diphosphate group. The latter usage has been approved, and two separate groups are now distinguished by being called 'biphosphate' [strictly bis(phosphate)] as in fructose 1,6-biphosphate.

Much of the difficulty can be avoided if recommendations for a self-consistent nomenclature are agreed at the very beginning of the study of a new class of compounds. This is the thought behind general principles for naming natural organic compounds, prepared by the Commission on Nomenclature of Organic Chemistry of the International Union of Pure and Applied Chemistry (IUPAC) and published as: Nomenclature of Organic Chemistry, Section F, IUPAC Information Bulletin, Appendix 53, 1976 (*Eur. J. Biochem.* **86**, 1–8; 1978).

The basic idea of Section F is to have a set of stem names for each group of natural products to which the general principles of organic nomenclature will apply. Thus, unsaturation and the presence, type and number of functional groups will be indicated by prefixes and suffixes according to the standard methods of Nomenclature of Organic Chemistry, Section A–D, stereochemistry according to Section E and isotopic substitution according to Section H. Modifications of a skeleton will be shown by methods first introduced in the Steroid Rules, and now set out generally in Section F.

Although Section F is designed to guide individuals so that they can themselves propose a clear and simple system of nomenclature for a new class of compounds, there remain great advantage in bringing together many workers in a field to agree on how its general principles should be applied. The Joint Commission on Biochemical Nomenclature (JCBN), joint between IUPAC and IUB (International Union of Biochemistry), set up in 1977, has, as one of its main tasks, the application of these principles to appropriate groups of natural products. In this it works closely with the Nomenclature Committee of IUB (NC-IUB).

In some areas recommendations for nomenclature have already been made. In others JCBN will be inviting specialists who are active in the biochemistry and chemistry of particular groups of compounds to form working parties to make such recommendations.

We hope that our colleagues will accept invitations to serve on such working parties and carry out an important task. We hope too that specialists in areas that need agreed nomenclature will offer to form working groups themselves. We would be glad to hear of suggestions for topics from any groups in such areas that already meet for discussion. We would be glad to hear of suggestions for topics that may need nomenclature agreements, even when not accompanied by an offer to serve on working parties!

P. Karlson

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We gratefully acknowledge the initiative of the late W Klyne in preparing this letter

Has food production kept up?

Sir,—John Gribbin has referred (16 November, page 208) to FAO data indicating that in the past two decades, while population has grown at 2% per annum food production has grown by an average of 2.8% per annum. As I have pointed out elsewhere (*Proc. Nutrition Soc.* **36**, 121; 1977) such aggregate figures are highly misleading Whereas, in the developing countries, the total increase in food production was 2.8% per annum in the 1960s, demand for food increased by 3.5% in those countries. Almost two-thirds of all developing countries showed a fall in self-sufficiency in 1970–72 as compared with 1961–63.

Even more critical is the situation in the food-deficit less developed countries, in which grain production increased by 2.5% per annum in 1960–74 but only by 1.7% per annum in 1967–74. Analysis by the International Food Policy Research Institute shows that even if grain production in those countries were to increase by 2.5% per annum to 1985, they would by then have a deficit of 100 million tonnes of grain per annum. The trend over the past four decades has been of an increasing surplus of grain in North America and an increasing deficit in the developing countries. The problem, therefore, is not that of overall world food production but of where the food is produced, and of its distribution, both between and within different countries, and also whether it is used to feed animals or human beings.

Tilo L. V. Ulbricht

Agricultural Research Council
London

Mr Moon's philosophy

Sir,—While I know very little about the ICUS of 1972 and 1973 and the organisation of Mr Haskell, it is ridiculous for Professor Nicholas Kurti (16 November, page 206) to place the ideas of Mr Moon on a par with those in Haskell's book "Full Circle: The Moral Force of Unified Science" where the latter postulates periodical tables for botany, zoology and sociology.

Professor Kurti seems to assume that he can simplify to the necessary degree to reach his conclusions situations which are extremely complex. The 'Give and Take' relation is a fundamental metaphysical principle and we are consciously or unconsciously dependent upon it. Mr Moon's philosophy asserts that the true unity exists in the Creator while in the creation there is only plurality even though it is ordered plurality. Mr Moon is concerned primarily with the order of life and with ordering life and he presents us with a conceptual apparatus which sets out many things to which we can relate in a gradual and ordered way.

I must explain that the ICUS meetings are not public relations ploys to further the Unification Church, nor are they frosting on Mr Moon's theological cake. Mr Moon founded the ICUS conferences because of his deep and sincere desire to set up a forum to further the striving of man to resolve "the most important problem of our time—the reconciliation of religion and science. The problem is not simply a matter of reconciling two academic disciplines. Rather the problem points to the need to investigate the dichotomy of facts (science) and values (religion and ethics). This separation of science and religion in the modern world has caused untold harm and serious damage to the development of humanity."

But while Professor Kurti himself says that the ICUS conferences should be judged by the contents of the published conference proceedings and not by selected remarks and others, his ideas about the teachings of Mr Moon's movement indeed echo the popular press. It would have been better for all concerned if Professor Kurti had consulted the official publications of Mr Moon's teachings and the activities of his movement.

Jane Salter

International Cultural Foundation,
44 Lancaster Gate, London W2

Laser progress?

Sir,—The Christmas laser display in Oxford Street, London has just given rise to the following exchange of conversation:

Scientist· "I have a laser almost as good as that in my lab."
Companion: "Yes! But is it in colour, or only black and white?"

C. B. Lucas

University of London, UK.

news and views

Echoes of the bat's cry

from Jennifer Altman and Shin-Ho Chung

As the basic mechanisms of hearing are beginning to be understood, auditory physiologists are turning their attention to the neural processing of complex acoustic signals. From several recent studies on amphibian (Pettigrew *et al. Nature* **272,** 138; 1978), avian (Knudsen & Konishi *Science* **200,** 795; 1978) and mammalian (Chalupa & Rhoades *J. Physiol. Lond.* **270,** 595; 1977) species, we now know that auditory space is accurately mapped onto a nucleus in the midbrain, while neurones in the higher cortical centres are specifically tuned to extract certain features of sound. As might be expected, those animals which rely mainly on their ears for survival have the most highly developed auditory brain, and slowly neurobiologists are unravelling the neural mechanisms by which such animals handle acoustic signals.

Of the several known species which construct a picture of the external world by listening to echoes from ultrasonic pulses, bats are the best known Each species of bat has its characteristic ultrasonic cry. Some, such as the little brown bat (*Myotis lucifugis*), use cries which are frequency-modulated, changing in pitch from high to low, whereas others, such as the moustache bat (*Pteronotus parnellii rubiginosus*), emit a cry of constant frequency, which tails off with a brief downward change in pitch. As the animal approaches the target, the emission rate of its cries increases, and the duration of each cry is shortened. From the echoes of its cries, the animal then gauges the size, distance and velocity, as well as the location, of the object ahead of it. Such an unorthodox way of seeing the external world is extremely efficient, as demonstrated by the agility with which bats avoid moving barriers (Jen & McCarty *Nature* **275,** 743; 1978), and the accuracy with which they catch their prey. It is estimated that a bat on a hunting spree catches about 500 insects per hour.

Several recent studies on the horseshoe bat (Möller *et al. J. comp. Physiol.* **125,** 217, 227; 1978) and on the big brown bat (Feng *et al. Science* **202,** 645; 1978) show how cells in the midbrain nuclei respond immediately to the distance of an object gauged by echolocation. In the auditory midbrain, there is a class of neurones which discharges only when an ultrasonic pulse mimicking the animal's cry is followed by a faint echo. These neurones are particularly sensitive to the delay between the pulse and echo, and different neurones are tuned to different ranges of pulse–echo delays; the optimal delay for eliciting discharges varies from 4.5 to 20 ms, which corresponds to target ranges from 0.75 to 20 m. Indeed, the entire midbrain appears to be elaborately designed to extract information from a pair of stimuli. Möller (*op. cit.*), for example, showed that the presentation of a first tone briefly enhances the sensitivity of the midbrain neurones, so that a faint echo following soon after can effectively be detected. The precise excitatory and inhibitory neural network underlying these observations remains to be elucidated.

In a series of studies on the moustache bat, Suga and his colleagues have demonstrated that a large part of the auditory cortex is devoted to detecting a slight shift of frequency components of the returning echo compared with the emitted cry (Manabe *et al. Science* **200,** 339; 1978; Suga *et al. Science* **200,** 778; 1978; Suga *Fed. Proc.* **37,** 2342; 1978). This Doppler shift contains information about the relative velocity of the animal and the target, and a remarkable behavioural adaptation enables the bat to utilise the specific tuning of its auditory neurones to the full. When the frequency of the returning echo is shifted to, for example, 63 kHz from the emitted frequency of 61 kHz, the bat reduces the pitch of its cry, so that the Doppler-shifted echo frequency would be at 61 kHz, the frequency to which the inner ear is specifically tuned (Suga & Jen *J. exp. Biol.* **69,** 207; 1977) and to which a disproportionately large area of cortical surface is devoted. In the primary cortex, neurones responding to frequencies from 61 kHz to 63 kHz are ordered concentrically, with the area excited by a 61 kHz tone located at the centre and the surrounding rings of cells excited by progressively higher frequencies. In addition to frequency selectivity, each neurone in this area responds only to a very restricted range of sound intensity. Lines of isoamplitude are at right angles to the isofrequency contours, and each neurone has both a best frequency and a best amplitude. When the echo is faint, neurones located on the lower part of the auditory cortex respond and these are excited by inputs from both ears. As the intensity of the echo becomes louder, neurones located on the upper part of the cortex begin to respond. These units are directionally sensitive, as they are driven by an input from the contralateral ear and inhibited by the ipsilateral ear. In this way, the brain encodes the location and the velocity of a target.

Further towards the centre, there is a cortical region which is devoted to processing frequency-modulated signals. Unlike the Doppler-shift region, this portion of the auditory brain has only a vague tonotopic representation, but contains instead clusters of neurones with similar response characteristics. The stimulus parameters to which neurones respond are more specific. Some neurones, for example, respond best when the frequency-modulated sound is followed by a faint overtone of the first sound. Others respond only to a pair of sounds with particular combinations of frequency, amplitude and timing, similar to the delay-specific

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cells in the midbrain. These neurones thus seem to act like feature detectors, and by comparing the spectral composition of echoes with the emitted signals, they may serve to detect the size and location of the reflecting object.

The study of the bat's auditory system is providing us with unexpected insights into how the brain is designed to utilise biosonar signals. The next task for researchers is to elucidate the intricate neural network that enables certain features of acoustic stimuli to be extracted, and the processes by which such connections evolve during development. Moreover, it will be of great interest to know how the middle ear of the animal is constructed so as to vibrate optimally at ultrasonic frequencies. Once this task is achieved, we shall be a step nearer to understanding the mechanisms of sensory processing and integration, for which the brain is most exquisitely designed.

Positrons at surfaces

from John Pendry

RADIOACTIVE sources provide a supply of positrons in the laboratory, and one of the applications of these particles is to studies of the solid state. In a recent Letter (Mills, Platzman & Brown, *Phys. Rev. Lett.* **41,** 1076; 1978) an important new advance in technique has been demonstrated: positrons can be controlled precisely enough to probe the surface of a solid on an atomic scale.

Conventional experiments use positrons straight from the source with energies of the order of an MeV, and fire them into the solid under study. Once inside, a positron interacts strongly with the electrons through Coulomb forces. Plasmons, collective oscillations of the electrons, rapidly dissipate the energy and in a very short time the positron has lost almost all its energy and momentum. Capture of the positron by its antiparticle, the electron, will eventually take place, producing a pair of γ rays with the disappearance of the positron and an electron, though the time for annihilation is long compared with the time to come to rest in the solid. By conservation of momentum the γ rays must have almost oppositely directed momenta, slight deviations from 180° being due to the relatively small but finite moments of the electrons with which the positrons annihilate. Measurement of the deviation, in experiments which look for coincidences of two γ rays, gives the momentum distribution of electrons in the solid, information which can be used to understand bonding of solids, and to measure the Fermi surfaces of metals.

An additional complication arises for insulators, where the positron's Coulomb potential is largely unscreened, and is strong enough to bind an electron into a hydrogenic state known as positronium. The positron will preferentially annihilate with its 'own' electron, and signals characteristic not of the solid, but of the positronium bound state, are seen.

Surface sensitivity in positron experiments came a step nearer when it was shown that the thermalised positrons can be re-emitted from a solid before they have time to annihilate. Unlike electrons, positrons tend to be repelled by many solids and if in diffusing around the solid they encounter a surface they will be expelled from the solid. The positrons do not acquire more than about 1 eV energy so are reasonably monochromatic and can be accelerated by electric fields to form a collimated beam with easily controlled energy (Canter, Mills & Berko *Phys. Rev. Lett.* **33,** 7; 1974).

The point is that positrons with only 100 eV kinetic energy have limited powers of penetration, typically a few tens of Å, and therefore can be used to probe surfaces on an atomic scale.

Mills, Platzman and Brown have performed the first experiments of this nature. They worked with clean, well characterised, single crystal surfaces of aluminium, silicon and chromium. The characterisation by LEED and Auger techniques is most important in surface experiments as contaminants can easily cover a surface and obliterate all of the clean surface properties. The energies of positrons re-emitted from the surface were analysed by applying a retarding field to the sample and measuring the increase in the number of positrons annihilating inside the sample. The spectra had two components: a sharp peak at very low energies due to positrons that had thermalised inside the solid, and a much broader distribution due to incompletely thermalised positrons. The latter component became more intense as the incident energy was lowered below 1000 eV, a consequence of decreasing penetrating power.

The sharp peak was seen in all the materials studied, beginning its rise at zero kinetic energy and having a width dependent on the solid: 0.5 eV for aluminium, 1.0 eV for silicon and 1.7 eV for chromium. Because the positrons in this peak have thermalised inside the solid, the peak cuts off at a maximum energy which is the 'negative affinity' the solid has for positrons.

Our understanding of positrons in solids and at surfaces is based on calculation and on some experimental annihilation rates and angular distributions. The new experiments will provide a more detailed picture of positron behaviour. Theories of the positron-solid force law such as that of Hodges & Stott (*Solid State Commun.* **12,** 1153; 1973) can now be checked, even to the extent of diffracting positrons from surfaces. The rate of energy loss of positrons in solids can be determined from the ratio of thermalised to unthermalised positrons emitted: accurate calculations are difficult especially when the positron is moving slowly. Some new problems present themselves. The width of the low energy emission peak is only of the order of 1 eV but this is still more than an order of magnitude larger than the thermal spread in energies. Simple theoretical arguments require that the positrons are accelerated normal to a perfect surface, and if inelastic collisions are excluded, emerge in a parallel beam with only a thermal spread of energies. Certainly this is the situation found for electrons in negative electron affinity systems. The emission process for positrons must be more complicated and is yet to be elucidated.

Finally, we can look forward to the more difficult experiment of detecting emission of positronium. Canter, Berko and Mills (*Phys. Rev. Lett.* **33,** 7; 1974) have shown that a large fraction of the positrons pair with electrons as they leave the solid and emerge as positronium. This pair-tunnelling process reminiscent of the interface between a normal and superconducting metal can be studied by measuring the angle and energy distribution of the ejected positronium. ☐

John Pendry is at the Daresbury Laboratory of the Science Research Council.

A hundred years ago

THE *Japan Mail* states that an astronomical observatory is to be established within the precincts of the Geographical Bureau of Tokio. The same journal also announces that telegraphic insulators, made at the village of Imari, in the province of Hizen, are of such good quality that they find large sale in Europe.

From *Nature* **19,** 19 Dec., 159; 1878.

Discrepancies in claims for endothermy in therapsids and dinosaurs

from R. E. H. Reid

L. S. Russell's claim, made in 1965, that dinosaurs were warm-blooded animals, has since been supported chiefly by the work of de Ricqlès on bone histology, and in papers by Bakker on other topics. The concept of dinosaurian endothermy has been welcomed by some authors, and represented as established in a popular book by Desmond; but such workers as Feduccia, Thulborn and Charig have remained unconvinced, and have severely criticised some of Bakker's claims. As usual some disputed issues authors are mistaken, while some of identical evidence, but one of Bakker's later arguments involves a serious factual error, unless many other authors are mistaken, while some of the arguments used in different papers are inconsistent or contradictory.

Bakker's later views appear in summary in his popular article 'Dinosaur Renaissance' (*Scient. Amer.* **232**, 4; 1975), in which late (Middle and Upper) Permian reptiles from southern Africa are said to be exclusively therapsids. These are interpreted as endotherms, and as living in cold post-glacial conditions, which restricted large ectotherms to the Permian tropics. They are restored as possessing hair as insulation, and as able to stay active in winter snows. Even Triassic climates were supposedly not warm throughout the year, and large thecodonts (erythrosuchids) from the early Triassic of South Africa were therefore probably also insulated endotherms. The relevance to dinosaurs is that thecodonts are the group from which dinosaurs originated.

These claims are consistent with de Ricqlès' conclusions from bone structure (in *Morphology and Biology of Reptiles* (eds Bellairs & Cox), 123 (*Linn. Soc. Symp. Ser.* **3**, no. 3, London, 1976)), but, unfortunately, not with other evidence. Charig points out (*ibid.* page **65**) that glaciation of southern Africa was not Permian but late Carboniferous, and that the late Permian reptiles include various non-therapsid types, some of which (pareiasaurs) reached large sizes. There was even a large labyrinthodont amphibian (*Rhinesuchus*). This information is easily available from well-known textbooks, such as Romer's standard *Vertebrate Paleontology* (third ed., Chicago University Press, 1966) or Haughton's *Geological History of Southern Africa* (Geol. Soc. S. Afr., 1969). Even stranger, Bakker's earlier analysis of predator–prey ratios, in a paper published in *Nature* (**238**, 81; 1972), shows more than one-third of the total prey biomass for the earliest late Permian zone (*Tapinocephalus*) as consisting of pareiasaurs, and describes its therapsids (deinocephalians), with pareiasaurs, as "almost certainly ectothermic". Later treatment of early therapsids as endothermic is within the bounds of reinterpretation; but what has become of the pareiasaurs?

Bakker also gives two opposite pictures of gait in therapsids. In his study of tetrapod locomotion (*Evolution* **25**, 636; 1971), he insists that their gait was always sprawling, and never even semi-erect; but, in 'Dinosaur Renaissance', even the earliest are said to have limb modifications which permitted trotting gaits. This leads to a further problem. In the earlier paper, Bakker argues that the 'fully erect gait' seen in dinosaurs is probably necessary for large endothermic tetrapods, because of the food-gathering requirements of endothermic physiology, and claims that the echidna *Zaglossus*, at 10 kg weight, "illustrates about the size limit possible for a homeothermic vertebrate with sprawling limbs." Large therapsids which he estimates at weights between 500-1500 kg in 'Dinosaur Renaissance' should hence have needed erect gaits as endotherms, if this argumen for dinosaurian endothermy is justified, but they cannot have had erect gaits if his analysis of limb movements is correct. In any case early forms seem unlikely to have had them.

The type of bone called 'endothermic' by Bakker is compact bone with a primary structure called fibro-lamellar by de Ricqlès (often, laminar bone *sensu* Currey), with more or less extensive Harversian substitution. This is now characteristic of large endotherms, and is seen by de Ricqlès (*op. cit.*) as reflecting their rapid initial growth rates, which in turn reflect high metabolic rates. A possible alternative correlation would be with size and gait only. It might, for instance, be claimed that bone formed from densely packed cylindrical primary osteons, with progressive Harversian remodelling as size and weight increase, is simply better adapted mechanically than lamellar-zonal ('ectothermic') bone as a structural material for the limb bones of large animals whose limbs support weight for long periods. It may even be needed for them to reach large sizes. This seems ruled out, however, as a primary correlation, if therapsid gaits are pictured conventionally, by the occurrence of this type of bone in early deinocephalians, and even the eotheriodont *Biarmosuchus*, but, ironically, this does not follow if even early forms possessed erect trotting gaits.

Treatment of therapsids as endotherms also weighs against this status for dinosaurs. Feduccia argued that impetus towards endothermy will have been greatest during periods of severe climate, and insufficient to lead to it in the times in which dinosaurs originated. Dodson's reply that "the ancestors of the dinosaurs lived at the same time and in the same place as the predecessors of the mammals" is valid if mammalian endothermy first arose in the immediate ancestors of the earliest 'true' (osteological) mammals. However, the precursors of the earliest therapsids were Lower Permian sphenacodonts. The earliest known thecodonts only date from the end of the Permian, long after large pareiasaurs had been able to enter southern Africa; dinosaurs did not appear until the Upper Triassic, when conditions presumably approached those of the Lower Jurassic optimum. Bakker claims Mesozoic climate as optimal at the end of the Jurassic, but marine invertebrates show minimal latitudinal zonation in Lias times, and a 'boreal' realm persisting from Bajocian times onwards.

It is not hard to find other minor inconsistencies. For instance, an estimated maximum sustained speed of 2.8 km h^{-1} for ostrich dinosaurs as ectotherms is contrasted with their having limbs like those of ostriches, which reach 50-80 km h^{-1} (*Nature*, 238, 81; 1972). Limb proportions however, are an index of speed, not endurance. In 'Dinosaur Renaissance', even early therapsids are supposed to have trotted, but examples (pristerognathids) are shown with semi-erect limbs, which would not permit trotting. Dinosaurs found within the Cretaceous Arctic circle in Canada are seen as suggesting the ability to tolerate cold, but large Cretaceous crocodiles from Saskatchewan are later pointed out as ectotherms. Such dinosaurs would also have needed to be migratory, unless able to feed in winter darkness. The predator–prey ratio for the early Cretaceous Cloverly Formation is based on the assumption that a 70 kg deinonychid preyed on two dinosaurs over 28 times heavier (2,000 kg) than itself, one of which was heavily armoured, and on a brachiosaur 571 times heavier (40,000 kg). This seems quite unlikely, unless it was simply a

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scavenger, whereas, the specialised claws of *Deinonychus* suggest active predation on animals about its own size.

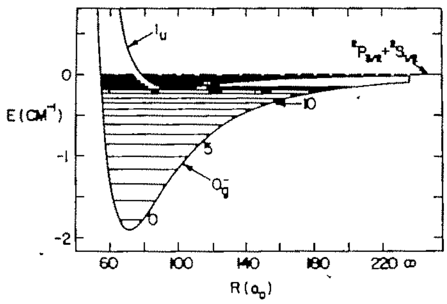
How do such problems affect the concept of dinosaur endothermy? They do not affect other arguments, such as Ostrom's contention that erect gaits may only be possible for endotherms, or Wheeler's recent comments (*Nature* **275**, 441; 1978) on cooling of the central nervous system. Erythrosuchids need not have been insulated, but did have whatever condition is responsible for 'endothermic' bone. The problem is that doubtful claims tend to discredit real evidence. But even so, if one follows Charig's rejection of predator–prey ratios, the only major line of evidence remaining is that from bone histology, which cannot be certainly attributed to endothermy rather than bulk.

I must also support Thulborn's view that small size, a naked skin, and endothermic physiology seems a lethal combination for hatchlings. This need not mean that dinosaurs were simply 'good reptiles', but their nearest possible approach to true endothermy, if their young lacked insulation, will have been some combination of fairly high metabolic rates with poikilothermy, (until temperature could be stabilised by bulk in the larger animals). Perhaps this type of physiology would be feasible for typically large animals, evolved in warm Middle to Upper Triassic conditions, but it does not correspond to the fully developed endothermy of mammals and birds, in which external insulation allows high stable temperatures to be maintained in small animals. ☐

Long-range molecules

from Graham Richards

Van der Waals forces are normally thought of as attractive. They are the weak attractions which hold molecules together with a binding energy often following the sixth power of the intermolecular separation. In fact this is only true for spherical charge distributions. Attractions of the c/r^6 form are appropriate for interactions between rare gas atoms but in more complicated instances this may not be true even between atoms. Perhaps most striking is the fact that if one of the atoms is in a spherically symmetrical S state and the other in an electronic state of P type then not only is the dispersion force of the form c/r^3 but it is repulsive!



The potential-energy curves for two long range states of Na_2.

This fact is utilised in a description of a new type of molecular electronic state by Stwally and Yea-Hwang Uang of the University of Iowa and Pichler of the Joint Institute for Laboratory Astrophysics in Boulder (*Phys. Rev. Lett.*, **141**, 1164; 1978).

The energy of a diatomic molecule is normally represented by a potential energy curve with a left-hand limit showing the vibrational turning point with the nuclei close together, and the right-hand branch the maximum stretch between the nuclei at the other extreme of vibration. Vibrational levels near dissociation give rise to 'long-range molecules' since although the molecule repeatedly vibrates classically from the inner turning point (typically 1 Å separation) to the outer one (typically 10–100 Å), it spends most of its time at the long range region.

The new type of pure long-range molecule is predicted to spend its entire time in long range regions with separations between 30 Å and 100 Å. These long-range molecules will be possible for electronic states where the repulsive van der Waals forces at large internuclear separations result in there being a small bump in the potential energy curve and a very small well in which vibrations can take place. The local energy minima at very long range arise from the recoupling of angular momenta.

In the familiar type of diatomic molecule potential curve the range of internuclear separation shown is perhaps 1–10 Å with the depth of the potential well being several electron volts (1 eV is approximately 8,000 cm^{-1} and 1,000 cm^{-1} is approximately 12 kJ mol^{-1}). This may be contrasted with the curves drawn in the figure.

Although typical in shape the depths of the potential well are now less than 2 cm^{-1} and average separation is over 30 Å (1 Bohr, $a_0 = 0.52917$ Å). The curves are those predicted by Stwalley *et al.* for two states of diatomic sodium.

Experimentally these states are visible as angular momentum recoupling extrema in the far wings of resonance lines of Rb and Cs. In particular, the red wing stellite on the 8521 Å line of Cs corresponds to the O^-_g state illustrated in the figure for Na_2. The authors hope to prepare molecular beams with significant populations of long-range levels of Li_2 and Na_2 and to observe rotational fine structure in bands involving the long-range states by laser spectroscopy. ☐

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Metallic hydrogen

from N. E. Cusack

A recent report by P. S. Hawke and fellow workers at the Lawrence Livermore Laboratory (*Phys. Rev. Lett* **41**, 994; 1978) that highly compressed hydrogen is a metal raises again one of the most interesting questions in the physics of condensed matter, namely, what decides whether a substance is metallic or not? Many theoretical and experimental studies, going back to classical papers by Mott in the 1950s, have made it clear that one decisive parameter is the density In a way this is obvious; at room temperature liquid mercury, for example, is metallic but its vapour is not. But whether there is a critical density at which a sharp phase change from metal to non-metal occurs and, if so, what electronic mechanism is at work are still open to discussion and much depends on whether the material is crystalline, or structurally disordered as in a fluid.

For crystals, it holds that if the bands of allowed electron energy are either fully occupied or completely empty, then the material is non-metallic Partially empty bands permit metallic conductivity and therefore, if a full and an empty band can be made by a density change to overlap in energy, they will both become partially full and a metal will be created. The reverse could occur if the density change caused two overlapping energy bands to separate These are called 'band crossing transitions' and are believed to occur in insulating iodine and metallic ytterbium which, under high pressures, reverse their roles and become a metal and an insulator, respectively However, other considerations enter for monovalent metals like sodium which have half full energy bands. If these could be reduced in density—not possible in the crystallised state—it is thought that at a critical density the electrons would condense on to the ions and be bound into neutral centres thus losing their freedom to conduct electricity. Contrary to the band crossing case, such a transition is chiefly an effect of the mutual interactions of the electrons themselves and is known as a Mott transition. There are theoretical reasons for believing that both Mott and band crossing transitions are discontinuous phase changes in crystals.

In fluids, or disordered solids, the whole matter is complicated by the structural chaos and although discontinuous transitions are not ruled out, gradual changes are quite possible. Furthermore, electrons may lose their ability to conduct electricity because the disorder itself confines them to finite regions of the material—an unexpected and subtle modification of the electron wave functions known as 'Anderson localisation'. Fluid mercury reduced in density to about $9\ \mathrm{g\ cm^{-3}}$ at about 1400 °C and 1500 bar pressure is believed to become non-metallic by a continuous process involving Anderson localisation.

In all this, the behaviour of hydrogen is particularly challenging for a number of reasons. In the first place, increasing the density of hydrogen sufficiently to give it any chance of becoming metallic requires enormously high pressures—in the megabar range. If the production of such high pressures is accompanied by high temperatures, knowledge of the sample density, or even of whether the hydrogen is liquid or crystalline, will require some understanding of the equation of state. Understanding of both the equation of state and the metal–non-metal transition ought to come relatively easily with hydrogen because it is the simplest element. However, solid hydrogen is a molecular solid and from the point of view of the electron energy band theory has full and empty bands and is therefore an insulator. If it is sufficiently compressed will it undergo a band crossing transition or will it suddenly transform to a monovalent metal with half full bands like the next simplest monovalent metals lithium and sodium? These questions have an interest beyond solid state physics because high pressure hydrogen exists in the larger planets of the Solar System, and planetary magnetic and other properties will be greatly influenced by whether their hydrogen layers are metallic or not.

In their paper, Hawke and collaborators reported high conductivity in a hydrogen sample at a density of $1.06\ \mathrm{g\ cm^{-3}}$ and a pressure of 2 Mbar. The detection of metallic hydrogen had been reported previously at about 2.8 Mbar by Grigor'ev *et al.* (*JETP Lett.* **16,** 201; 1972) but by interpreting an 'anomaly' in the thermodynamic properties rather than by observing high conductivity. These two groups attain the high pressure by causing an explosive to implode a cylindrical container. In the Livermore experiment the imploding steel cylinder compresses magnetic flux which in turn compresses the hydrogen sample inside its silver holder. In such experiments measurements must be carried out by rapid methods during the 10 or 50 microseconds available. Densities, for example, are found by observing the sample volume by X-ray flash radiographs, and conductivities by operating continuously a reflectometer using 15 MHz pulse trains. Other investigations of high pressure hydrogen have tried shock waves and high intensity laser pulses to produce the pressure. It is not impossible to get into the megabar range with static anvil devices and indeed Vereshchagin *et al.* (*JETP Lett.* **21,** 85; 1975) claimed in 1975 to have detected metallic hydrogen at 1 Mbar in such a static equipment with the sample at 4.2 K.

There seems little residual doubt that metallic hydrogen can be made and that it needs a small number of megabars to do it. Theoretical estimates of the transition pressure have ranged in the past from about 1 to 20 Mbar and it is of course no easy matter to calculate it. The theoretical approach is to calculate the energies of the atomic metallic and molecular insulating phases separately using solid state methods and then determine the pressure and density at which the free energies of the two phases are equal. One recent calculation (Ramaker *et al. Phys. Rev. Lett.* **34,** 812; 1975) gave 2.1 Mbar which suggests that theory and experiment are beginning to converge. Hawke *et al.* do not consider that a band crossing transition in the molecular crystal has been ruled out by their work but that an abrupt transition to an atomic phase comparable with metallic sodium is at present favoured.

The interest of this work to physicists of condensed matter is considerable and is enhanced both by a suggestion of Ashcroft's (*Phys. Rev. Lett.* **21,** 1748; 1968) that metastable metallic hydrogen (if it exists) might be superconducting at room temperature, and also by the relevance of compressed hydrogen isotopes to nuclear fusion. A growing understanding of this interesting example of a metal–non metal transition can be confidently expected but experiments and theory are both difficult and progress may not be rapid. ☐

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The cladistic revolution—can it make the grade?

from L. B. Halstead

OVER a decade ago a German entomologist, W. Hennig, published his book *Phylogenetic Sytematics* (University of Illinois Press, Urbana, 1966) in which he presented a codified methology for determining the relationships of organisms. As the entire system was designed in such a way that fossils could hardly be included, little serious note was taken of the phenomenon which has become known as cladism. Palaeontologists did not reckon on the ingenuity of certain colleagues, who not only succeeded in applying it to fossil fishes, but managed to convert others to this new system.

It was with this background in mind that a special session was set aside for the topic to be debated at the 26th Symposium of Vertebrate Palaeontology and Comparative Anatomy held in September at the University of Reading.

P. Forey, British Museum (Natural History), outlined the methods and aims of the cladists. Hennig had stressed that it was necessary to distinguish primitive or original characters, which he termed plesiomorphic, from advanced or derived ones, now named apomorphic, in order to ascertain the direction in which evolution had progressed. If a number of lineages can be united on their primitive or plesiomorphic characters the grouping is termed paraphyletic and as such is rejected in the Hennigian scheme, since only strictly monophyletic lineages are recognised. Groups that are united on shared derived characters, or a synapomorphy, can, however, be placed in a valid taxon. The approach of the followers of Hennig is best seen in their construction of cladograms, a system of repeated dichotomies where at each branching a new monophyletic lineage is distinguished by a change in at least one derived character state from the original condition. It is axiomatic that such sister groups are given identical rank. Hence an adjacent lineage and all those subsequently descended from it will have the same taxonomic rank as the preceding lineage. Furthermore there is no place for ancestral species in a cladogram. Where complete sequences from species to species or, as G. MacIntyre (Queen's College, New York) demonstrated, from genus

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to genus can be demonstrated, then since they will represent a monophyletic lineage, they must necessarily be included in a single specific taxon.

In order to construct a cladogram it is necessary first to be able to distinguish between original and derived characters and to assemble them in some kind of hierarchy, which as S. Westoll (University of Newcastle) stressed, involved a degree of subjectivity from the very outset.

A cladogram is essentially an hypothesis and its validity is continually tested by the law of parsimony (that no more causes or forces should be assumed than are necessary to account for the facts). An example of this process was illustrated by cladograms presented by P. Janvier (University of Paris) on agnathans and B. Gardiner (Queen Elizabeth College, London) on choanichthyans both of which were demolished within hours of their being proposed. One of the problems of cladism is that fossils can only be considered once the model for their modern representatives had been worked out — a very difficult situation for groups with no agreed living representatives such as heterostracan agnathans and dinosaurs.

Cladism has been extensively applied to insects and the symposium volume edited by Greenwood, Miles and Patterson (*The Interrelationships of fishes*, Academic Press, London, 1973) ranks as a major monument to the application of Hennigian principles to the study of fish.

In contrast the very concept of structural grades is anathema to all cladists Bonde (*Colloque internat. CNRS* No. 218, 293; 1975) claimed "very little has been achieved in studies of other vertebrate groups, for example by Romer (1966, 1968) and Halstead (1969) where grade classification and paraphyletic groups are repeatedly recommended because of the enormous influence of Simpsonian philosophy." Yet as all teachers and writers concerned with the study of the history of life on this planet well recognise, major evolutionary events can be best understood in terms of major structural grades, which happen to coincide with the major classes of the vertebrates. The idea of separate evolutionary lineages achieving a higher grade of organisation, say from a reptilian to a mammalian grade, should occasion no surprise. In terms of understanding evolution, the fact that many parallel changes can be related to adaptations to similar conditions, is more significant than listing trivial trademarks from which cladograms can be constructed. The aim of the cladists is simply to delineate relationships and to insist on a classification that reflects this in its entirety at the expense of all other considerations.

Yet if it were possible to trace the ancestry of man back in time to a rhipidistian fish then the entire sequence of stages would be by definition a single monophyletic species. This must surely have happened, yet most zoologists and palaeontologists would surely accept the reasonableness of dividing such a lineage into a number of arbitrary divisions. Commonsense alone would dictate such a course of action. Even though it is possible to take all the end twigs of the evolutionary tree that exist at the present time and construct a hypothetical tree of them, such sharp distinctions must of necessity vanish as they are traced back in time Evolution is a gradual process that takes place through time and man simply imposes on it his own arbitrary system of classification.

The issue was summed up for many by R. Parrington's (University of Cambridge) exasperated exclamation that according to the cladists a lungfish is more closely related to a cow than to a salmon to which C. Patterson (British Museum (Natural History)) answered "Yes, I cannot see what is wrong in that".

The cladists adhere to the tenets of Hennigism with religious fervour and are already entrenched in some of the major museums in the world. The new evolution and diversity exhibit in the Natural History Museum is destined to be organised to show relationships. If the familiar arrangement of family trees illustrating not only relationships but also their distribution through geological time were to be replaced by elaborate cladograms, I feel it would be a severe error of judgement Cladograms are difficult enough for experts in the field to comprehend fully, let alone the casual visitor seeking either knowledge or entertainment. ☐

Earthquakes and fault gouge

from Peter J. Smith

MANY large shallow earthquakes are evidently associated with slip along pre-existing faults in the Earth's crust But this is not to say that the shallow-earthquake mechanism is anything like fully understood; on the contrary, there are outstanding problems of a quite fundamental nature. For example, laboratory experiments on the frictional sliding of rock carried out during the late 1960s suggested that the shear stress needed to activate rock-on-rock sliding was an order of magnitude higher than that actually present on the San Andreas fault, the latter being deduced from local heat flow studies In other words, stick-slip seemed the most likely mechanism for shallow earthquakes, but it was by no means clear that, in the San Andreas fault zone at least, the conditions for it actually obtained.

More recently the stress criterion has been relaxed insofar as Hanks (*Pageoph.* **115**, 551; 1977) and others have argued for a real stress along the fault rather higher than that previously supposed. Even so, it still seems worthwhile to consider what, if anything, could reduce the shear stress actually required to produce fault slip. An obvious possibility here is the presence of fault gouge, for repeated movements in the highly fractured San Andreas fault zone are likely to have led to extensive comminution, and the resulting gouge may even have been altered by hydrothermal fluids Presumably, therefore, this gouge, altered or not and dry or wet, could modify the San Andreas fault's slip behaviour Indeed, some very recent laboratory experiments (for example, Summers & Byerlee, *Int. J. Rock Mech. Min. Sci.* **14**, 155; 1977) have shown that artificial gouge between cut rock surfaces often reduces the coefficient of friction, which makes it all the more surprising that gouge—a long-known substance, after all—has not hitherto played a much greater part in mechanism studies. Yet as Wang *et al.* point out (*Geophys Res. Lett.* **5**, 741; 1978), "Experimental data on gouge under conditions appropriate for upper and mid-crustal environments are still lacking".

But first, does fault gouge actually exist in appreciable quantity at depth in the San Andreas fault zone? Intensely faulted and severely altered rocks combined with clay minerals are certainly present at the surface, even though the lack of detailed mapping and classification makes it difficult to obtain 'representative' samples Nevertheless, Wang and his colleagues have collected fault gouge from outcrops along both the San Andreas and Hayward faults and, for reason that will soon become clear, have measured P and S seismic wave velocities in them at various confining pressures up to

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Sicily: the world reservoir for thalassemias and haemoglobinopathies

from Gino Schilirò

SICILY is particularly affected by the hereditary disorders of haemoglobin synthesis (the thalassemias and haemoglobinopathies) that are found all around the Mediterranean. In Sicily the very common β-thalassemias and the many haemoglobinopathies present a serious public health problem. For this reason a Centre for the study and treatment of thalassemias was established in Catania in 1965. This Centre, under the direction of Professor Giuseppe Russo, staffed by physicians, laboratory technicians and nurses, serves not only as a modern treatment facility and follow-up for 250 children with Cooley's anaemia (thalassemia major) but also as a centre of research into thalassemia and of a public education campaign[1]. Mass screening to detect thalassemia carriers and those with other haemoglobinopathies, and preventive genetic counselling is an important element of the programme of the Centre. Since the estimated number of Sicilian newborns with Cooley's anaemia is about 105 each year, we are developing a programme for prenatal diagnosis.

From 1974 to November 1978, 7,143 subjects with suspected hereditary haematological disorders were examined at the Centre: 28.5% of them were carriers of haemoglobin anomalies; 292 were homozygous β-thalassemia, 1,342 β-thalassemia trait and four homozygous $\delta\beta$-thalassemia. We also found 40 cases of α-thalassemia as α_1, α_2 and HbH disease, but no cases of hydrops fetalis have been seen. The exceptionally high incidence of the haemoglobinopathies in Sicily is reported in Table 1. The interaction between the thalassemias, with their genetic heterogeneity, and the haemoglobinopathies gives extremely interesting haematological pictures[2]. If one considers that several abnormal haemoglobins have also been reported by others[3] in Sicilians or people of Sicilian ancestry, then it is evident that Sicily, because of its geographical position and history, is a place of unique genetic admixture.

In studies of globin synthesis in randomly chosen patients with Cooley's anaemia, two groups have been distinguished: β^0-thalassemia (30%) characterised by the absence of β chains, and β^+-thalassemia (70%) with low levels of β chain synthesis[4]. In collaboration with other Italian and foreign laboratories, research at the Centre has contributed to the clarification of some of the molecular defects of the thalassemias. $\delta\beta$-Thalassemia has been found to be caused by a genetic delection[5]. We have also observed that while β globin synthesis is completely absent in cells of patients with β^0 Catania, there is β-globin mRNA in the cytoplasm of the cells of these patients and that the β^0 patients seem to have the normal number of β-globin genes[6].

Haemoglobin S is the most common abnormal haemoglobin found in Sicily. The mean frequency is 1% although in certain areas it may reach 14%, as in people of African origin. The chemical and physical identity of Hb S in both USA Blacks and Sicilians as well as the presence of African blood group markers suggest that Hb S may have spread from Africa to Sicily[7]. Another haemoglobin we have found is Hb C, of known African origin. Among the 18 families with Hb J Oxford reported in the literature, 13 are Sicilian or of Sicilian ancestry. Carriers of this haemoglobin are asymptomatic and discovered only by screening. It should be noted that in all our cases the rate of Hb J Oxford was about 25%, supporting the hypothesis that α chain production is controlled by four genes. A case of interaction between homozygous β^0-thalassemia and Hb J Oxford, producing a new alkali-resistant haemoglobin $\alpha_2{}^J\ \gamma_2{}^F$, is an example of the wide range of possible genetic combinations in this island with so many disorders of haemoglobin synthesis[8].

Although these data come from a selected population at risk, the phenomenon still remains extremely important. In fact, since 1893 many people have emigrated from Sicily to North and South America, to Australia and, more recently, to North Europe. Especially if one considers that, in the 20 years between 1951-71, 1,010,963 Sicilians emigrated, the Sicilian haemoglobin abnormalities become of general interest.

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Abnormal haemoglobins found among 7,143 blood specimens

Genotype	Number	Structural* alteration	Genetic combinations found (number of cases)
Hb S	277	β^6 Glu → Val	S–S(8) S–A(194) S–β^0Th(30) S–β^+Th(55)
J-Oxford	20	α^{15} Gly → Asp	J–β^0Th(1) A–J(19)
Lepore Boston	17	α_2 $(\delta^{87}\ \beta^{116})_2$	I–βTh (5)
Hb C	10	β^6 Glu → Igs	A–C (8) C–β^+Th (1) (C–β^0Th) (1)
J-Baltimore	9	β^{16} Gly → Asp	J–β^+Th (1) A–J (8)
G-San Josè	3	β^7 Glu → Gly	G–β^0Th (1) G–α_1Th (2)
D-Punjab	3	β^{121} Glu → Gln	D–A (3)
Shepherd's Bush	2	β^{74} Gly → Asp	S B–A (2)
G-Copenhagen	1	β^{47} Asp → Asn	G–A (1)
Incompletely characterised	2		

*Characterisation of the abnormal haemoglobins was performed by L. Tentori and coworkers at the Centro di Riferimento per le Talassemie ed Emoglobinopatie in Rome.

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[1] Schiliro *et al. Lancet* **i**, 598 (1976).

[2] Russo *et al. Acta haemat.* **28**, 329 (1962); *Blood* **42**, 763 (1973); *Pediat. Res.* **11**, 1019 (1978); Musumeci *et al. J. med. Genet.* (in the press).

[3] See for example Sansone *et al. Acta haemat.* **43**, 40 (1970); Ricco *et al. FEBS Lett.* **39**, 700 (1974); Sharma *et al. Biochim. biophys. Acta* **393**, 379 (1975).

[4] Schiliro *et al. Acta haemat.* **60**, 193 (1978).

[5] Ottolenghi *et al. Cell* **9**, 71 (1976).

[6] Ramirez *et al. Clin. Res.* **24**, 442A (1976); *Nature* **263**, 471 (1976).

[7] Sandler *et al. Acta haemat.* (in the press).

[8] Schiliro *et al. Blood* **48**, 369 (1976).

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2 kbar (equivalent to a depth in the Earth of 8-10 km).

The results are simply stated. The P wave velocities in the fault gouge samples and their variation with pressure (depth) are very similar to those obtained by Healey and Peake (*Bull. Seismol. Soc. Amer.* **65**, 1177; 1975) for the central Californian region of the crust bounded by the San Andreas and the San Benito faults. Moreover, both the P and S wave velocities in the gouge and the P wave velocities obtained by Healey and Peake are significantly lower than those in intact rocks, whether the intact rocks be *in situ* crust in the region outside the San Andreas fault zone or samples brought from the San Andreas fault zone itself (Lin, thesis, University of California, 1977). In other words, the low seismic velocities in the San Andreas fault zone are consistent with the presence of appreciable amounts of gouge to depths

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of at least 8-10 km. Support for this interpretation also comes from gravity data, for to explain the steep gradient in the Bouguer gravity profile near the San Andreas fault zone it is apparently necessary to invoke the presence of a slab of low density (~2.6 g cm^{-3}) extending to a depth of 8-10 km. A density of 2.6 g cm^{-3} is typical of fault gouge.

Wang and his coworkers go no further with their experiments; but they have gone far enough to show that fault gouge as a possible modifier must not be ignored when it comes to considering the causes of shallow earthquakes. □

Phase transitions in spin glasses

from a Correspondent

IF the temperature of a supercooled liquid is lowered sufficiently, it may form a glass. For twenty years or more there has been an inconclusive debate as to whether this change is of purely kinetic origin, occurring when the relaxation times of the liquid become longer than the patience of the experimenter, whereupon the liquid appears to have solid properties, or whether the change reflects an underlying phase transition. It looks as though the debate on the causes of the similar phenomena found in spin glasses may be just as lengthy and inconclusive.

A spin glass is typically a substitutional alloy of a few percentage of a transition metal such as iron or manganese in a host metal such as copper or gold. The transition metal impurities—the spins of the spin glass—can be taken as being approximately randomly distributed throughout the alloy. They interact with each other through the conduction electrons of the host metal to give a long-range coupling between the spins whose sign depends on the distance between the spins. This means that the interaction between some spins is ferromagnetic in sign which favours their parallel alignment while that between other pairs of spins is antiferromagnetic which favours their antiparallel alignment. The net effect of such a mixture of competing interactions is to produce a ground state in which the orientation of the spins is chaotic and which has no resultant magnetic moment.

When cooled below its freezing temperature T_F, the properties of a spin glass are radically altered in much the same way as the properties of a supercooled liquid are altered below the glass transition temperature T_g. Above T_F an induced magnetic moment rapidly decays away on a microscopic time scale when the magnetic field which induced it is switched off. Below T_F an induced moment can take hours to decay away. One can immediately deduce that some relaxation processes in a spin glass take place very slowly. Another characteristic is the 'cusp' in the magnetic susceptibility at the freezing temperature. The susceptibility as a function of temperature rises at T_F to a maximum which is quite sharp, especially when the susceptibility is measured using small applied fields.

Non-analytic behaviour such as a cusp usually indicates that a phase transition must be taking place. In 1975, Edwards and Anderson (*J. Phys.* **F6**, 1927; 1975) proposed a phase transition theory for spin glasses which gave a seemingly satisfactory explanation of the cusp. The order parameter q of their theory is the probability that any given spin is still pointing after an infinitely long time in the same direction as it was at some initial time. Above T_F, q is zero, but below T_F, which is identified as the phase transition temperature, it is non-zero and increases to unity as the temperature approaches absolute zero.

Although we live in a three-dimensional world, it is both interesting and useful to examine (theoretically!) the nature of a phase transition in worlds of different dimensionality. There is always a 'lower critical dimension' below which the phase transition will not take place. Thus the Heisenberg type of ferromagnet does not have a phase transition in two dimensions for its lower critical dimension is two. The lower critical dimension for the spin glass transition is controversial, with arguments being advanced for two, three and four dimensions. Numerical evidence derived from high-temperature series expansions seems to favour four (Reed, *Phys. Lett.* **A68**, 473; 1978). If that really is the case, then the phase transition theory of spin glass behaviour fails and an alternate explanation must be sought.

An explanation which actually predates the phase transition theory is that spin glass behaviour is entirely a non-equilibrium effect resulting from the long relaxation times which exist within spin glasses at low temperatures. The relaxation modes probably involve activation processes in which a potential barrier separating two easy orientations of a spin has to be surmounted. This will produce relaxation times which follow an Arrhenius law and so rapidly increase at low temperatures. The 'cusp' in the susceptibility is explained on this hypothesis by observing that at temperatures below T_F only a fraction of the total spins present will contribute to the susceptibility, namely those spins or groups of spins which can relax on a time scale shorter than the time scale of the experiment. The other spins are effectively frozen in fixed orientations and do not contribute. For a given experimental time scale, the further the temperature is lowered below T_F the larger the fraction of frozen spins becomes and hence the smaller will be the observed susceptibility. A corollary of this hypothesis is that the freezing temperature T_F should alter with the experimental time scale. Lengthening the time scale should enable more spins to contribute to the susceptibility and so depress the apparent freezing temperature. A similar effect occurs at the glass transition in ordinary glasses where T_g falls when the liquid is allowed a longer time to come to equilibrium.

A recent experiment of Murani and Heidmann (*Phys. Rev. Lett.* **41**, 1402; 1978) clearly indicates that lengthening the time scale depresses T_F. They have carried out neutron inelastic scattering measurements on a Cu–8 at % Mn alloy at three different energy resolutions. The temperature dependence of the elastic magnetic cross section can be extracted from the data. It is related to the Edwards-Anderson order parameter q on a phase transition hypothesis and to the number of frozen spins on a relaxation time hypothesis. The elastic cross section increases below a certain temperature but the temperature at which this happens, the nominal freezing temperature, varies with the energy resolution employed. They argue that the time scale in their experiment is proportional to the inverse of the energy resolution. For a time scale of 10^{-11} s, T_F is 75 K, whereas T_F is about 40 K on a time scale of 10^{-2} s (this latter data is from separate experiments on the a.c. susceptibility). At first sight this appears to be strong evidence against a phase transition (since a phase transition should take place at a unique temperature) and in good accord with the explanation of spin glass behaviour in terms of long relaxation times and frozen out spins.

Unfortunately it is not as simple as this. A phase transition could be accompanied by long relaxation times which would produce an apparent freezing temperature that decreased with increasing experimental time scale, but which eventually saturates at the true transition temperature T_F for a hypothetical experiment with an infinitely long time scale. There is just a hint of such a possibility within their data but it is not sufficiently strong to be compelling evidence. It is clearly going to be very difficult to decide from experiments of this kind whether spin glass behaviour is or is not due to a phase transition. A new idea is needed for a conclusive experimental test to distinguish the rival approaches.

review article

Electron cooling and its applications in elementary particle physics

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In recent years, experiments in high-energy physics have shown that one of the most promising means of studying the properties of elementary particles is by colliding beams. First, they open up a region of interaction energies at present unattainable using conventional accelerators. Second, they allow experiments to be performed in extremely clean conditions, when the observed interaction between two colliding particles is not contaminated by the presence of secondary interactions as in the conventional particle accelerator–fixed target scheme. This article describes the electron cooling method applied to proton–antiproton colliding beams.

THE first experiments carried out with electron–electron colliding beams in 1965 at Stanford and Novosibirsk gave a convincing demonstration of the potential of this technique in high-energy physics. In these experiments, from practical considerations, electrons were preferred, however, to obtain more information on elementary particles, colliding beams of particles and an anti-particles are of much greater interest. In the interaction between a particle and an anti-particle, a state is produced in which the total charges of all kinds, such as electrical, lepton, and baryon, equal zero, and the system is then released from the conditions imposed by the conservation laws of these charges. In fact, this state is equivalent to a pure energy-blob, capable of creating any particle–antiparticle pairs permitted by the energy-momentum conservation law. The first experiments on colliding beams with particle–antiparticle (electron–positron) annihilation were carried out at the Nuclear Physics Institute in Novosibirsk on the VEPP-2 apparatus (abbreviations of Vstrechnye Elektron-Pozitronnye Puchki—electron–positron colliding beams). The energy of the particles in these experiments reached 0.67 GeV. There are six electron–positron colliding-beam devices at various accelerator centres throughout the world (Novosibirsk, Stanford, Orsay, Hamburg, Frascati), with energy ranges from 2×0.2 to 2×4 GeV. Three more electron–positron colliding beam machines are under construction (Novosibirsk, Hamburg and Stanford) with an energy range of 2×4 to 2×20 GeV.

Experimental constraints

Electrons and their antiparticles are interesting as they represent 'point' objects: according to current experimental data the size of these particles does not exceed 10^{-15} cm. Unfortunately, for electrons and positrons the practical energy limit for colliding beams in their traditional configuration is not very far from the level attained today. This limit is associated with synchrotron radiation, the intensity of which increases as the fourth power of the energy of the particle, and decreases only as the first power of the orbit radius, so that increasing the size of the ring provides no real solution to the problem. Colliding beams of heavy particles do not have this problem as the intensity of synchrotron radiation is inversely proportional to the fourth power of the rest mass of the particle. But here, unhampered by energy losses, we have at the same time lost a mechanism which quenches the oscillations of the particles in the storage ring and so permits multiple storage of particles in the same phase volume.

For proton–proton colliding beams this does not in principle create any constraints as there are ways of producing intense beams of protons. The first proton intersecting storage rings have been in operation at CERN since 1971. However, for antiparticle beams accumulation is essential because of the low production yield. Antiparticles are generated with a large phase volume and it is therefore important to incorporate a mechanism for reducing the phase volume of the beam.

Such a mechanism was found by Budker, for heavy particles, who proposed the electron cooling method. Subsequently, it was realised that this method permits the accumulation of large antiproton currents and allows experiments to be carried out on proton–antiproton ($p\bar{p}$) collisions (A. Skrinsky). Hence, in 1966 at the Paris conference on colliding beams[1], in addition to the electron cooling method, Budker presented a $p\bar{p}$ colliding beam project worked out at our Institute. Although both the electron cooling method and the $p\bar{p}$-colliding beam project evoked great interest little progress was made, for the following reasons. First, the electron cooling method had not been demonstrated experimentally. It seemed to be an exotic idea with little hope of realisation. Second, during the 1960s, experiments on $p\bar{p}$ colliding beams were considered an important but extremely complicated way of supplementing $p\bar{p}$ experiments studying the nature of strong interactions.

Today the situation has changed radically. The observation of point-like constituents in protons which are now identified with quarks, has forced us to reconsider our attitudes to $p\bar{p}$ colliding beams: from the point of view of the quark model of strongly interacting particles, such beams are equivalent to colliding

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beams of quarks and antiquarks. This implies that $p\bar{p}$ colliding beams provide fundamental information similar to that obtained from electron–positron (e^-e^+) colliding beams. During the interactions, energy is liberated 'at a point' that is it is not smeared over an extended object; which allows information to be obtained on the properties of matter at limitingly small distances. According to current ideas, the proton (antiproton) contains three quarks, so that $p\bar{p}$-colliding beams with a given energy are equivalent to e^-e^+ colliding beams with energy three times less.

Finally, during the past few years, a decisive step forward has been made towards the creation of $p\bar{p}$-colliding beams: the electron cooling method has been studied and developed experimentally. This article describes this method and its potential for elementary particle and nuclear physics.

Machine luminosity

The efficiency of a colliding beam machine is characterised by the luminosity L, a quantity equal to the number of interactions per unit time during the collision of two beams per unit interaction cross-section. Then the counting rate in the i-th reaction channel with cross-section σ_i is

$$\frac{dN_i}{dt} = L\sigma_i \tag{1}$$

In the case of two bunches with N_+ and N_- colliding particles the luminosity is given by the relationship

$$L = \frac{N_+N_-}{S} f \tag{2}$$

where f is the frequency of revolution of the particles in the ring, and S is the cross-section of the larger of the bunches. The fundamental problem with colliding beams is to obtain the maximum possible luminosity. We note that equation (2) holds if the detector sees the whole intersecting region. Hence it is advantageous to work with beams of short bunches.

When working with antiparticle beams the problem of intensity is especially serious. Antiparticles are produced by accelerators in comparatively small quantities, being ejected from a target by a beam of particles of sufficiently high energy. Moreover only a small fraction of the antiparticles thus generated can be injected into the storage ring: the permissible energy spread in a beam of anti-particles is usually a fraction of a per cent of the mean energy of the injected particles. For example, in the VEPP-2M storage ring out of 10^{11} electrons it was possible to obtain only of the order of 10^7 useful positrons, while for experiments with colliding beams it is necessary to have at least 10^{10}–10^{11} particles. Thus the only course is the accumulation of antiparticles: by multiple injection into the same phase volume of the storage ring. For this, it is necessary to constrict the phase volume of the injected beam before the next injected pulse, thus releasing phase space for the next lot of particles. This increase in the density of the beam in phase space takes place if friction is introduced into the system of particles.

Particle oscillations

A particle orbiting in a storage ring is known to perform oscillations in the focusing fields of the ring magnets with respect to some equilibrium trajectory. Three-dimensional oscillations in the (x, y, z) coordinate space correspond to motion over the surface of a six-dimensional ellipsoid in the coordinate-momentum (p_x, p_y, p_z, x, y, z) phase space. The set of N particles occupies some volume in phase space which remains invariant with time if the oscillations are not damped. If, however, a frictional force acts on the particles, the amplitude of their oscillations decreases with time, and, consequently, the volume which they occupy decreases.

If the frictional force $\mathbf{F}$ is proportional to the total velocity $\mathbf{V}$ of the particle

$$\mathbf{F} = -\lambda m \mathbf{V} \tag{3}$$

the decrease of the amplitude (A) of oscillations in the case of weak friction is exponential ($\lambda \ll \omega$), where m and ω are the mass and frequency of the oscillations of the particle, with a decrement

$$\lambda = \frac{-1}{A}\frac{dA}{dt} \tag{4}$$

In addition to friction, diffusion processes are always present in a real system, the action of these may be characterised by the diffusion coefficient D:

$$\frac{da^2}{dt} = D \tag{5}$$

Then the damping of the oscillations and the constriction of the phase volume reaches some steady equilibrium value when friction and diffusion are equal:

$$a_e^2 = \frac{2D}{\lambda} \tag{6}$$

These equations define two important characteristics of the storage ring: the rate of compression of the phase volume λ and the minimum (steady) dimension a_e of the stored beam, on which the luminosity of the storage ring depends.

Radiation friction

An example of such a dissipative process is the radiation friction for electrons and positrons due to synchrotron radiation which we have already mentioned. The force of reaction of the radiation is directed opposite to the total velocity of the particle. If the accelerating system of the storage ring keeps the longitudinal component of momentum constant on average, then the radiation friction quenches the deviations of the momentum from the equilibrium value. Radiation friction is a powerful dissipative process: typical damping times of the electron and positron oscillations in operational accelerators are from 1 s down to milliseconds. This allows the problem of multiple stacking to be solved. Hence electron–positron colliding beams have high luminosities. This mechanism, as noted above, does not apply to heavy particles—protons, antiprotons or ions.

Electron friction

In Budker's method, the friction in a beam of heavy particles circulating in a storage ring is introduced by the flow of electrons. For this, it is necessary to arrange in the straight section of the trajectory of the heavy particles, their interaction with electrons of the same mean velocity as the heavy particles. A particle whose velocity differs from the mean experiences, as a result of collisions with the electrons, the action of a frictional force which tends to damp its oscillations. In the simplest case of homogeneous electron beam with maxwellian velocity distribution the damping for each of the three spatial degrees of freedom is identical:

$$\tau = \frac{1}{\lambda} \approx \frac{0.5}{r_e r_p c L}\frac{\gamma^2}{n_e \eta}\left[\left(\frac{T_e}{mc^2}\right)^{3/2} + \left(\frac{T_p}{Mc^2}\right)^{3/2}\right] \tag{7}$$

Here r_e, r_p are the classical radii of the electron and the heavy particle, m, M, T_e, T_p are their masses and temperatures, n_e is the electron density in the cooling current, $\gamma = (1-\beta^2)^{-1/2}$ is the relativistic factor, β is the mean velocity of the particles in units of the velocity of light c and η is the fraction of the perimeter of the orbit occupied by the cooling section.

This model is equivalent to a homogeneous two-component plasma (particles–electrons), while equation (7) is the relaxation time of the plasma (equalisation time of the temperature of both components). To find the steady amplitudes of oscillations, equation (6), in this simple case there is no need to calculate the diffusion coefficient for a heavy particle in the electron gas: it is clear that the steady state corresponds to the equilibrium temperature of both components:

$$T_p \simeq T_e, \text{ or } V_p = \left(\frac{m}{M}\right)^{1/2} V_e \qquad (8)$$

Thus if the temperature of the cooling electrons is low ($T_e \ll T_p$), electron cooling works effectively. (All units are given in the centre mass rest frame.)

Naturally, the kinematics of a real cooling process are more complicated. The detailed motion of the proton beam in the storage ring must be taken into account, and also the effect of errors in the equalisation of the mean velocities of electrons and protons, coherent fluctuations of the electron beam, and so on.

Electron beam cooling

In the experiments described below, various effects associated with special features of the electron beam have been studied: the role of the sharp anisotropy of the velocity distribution function of the electrons in a beam accelerated by an electrostatic field[2], and the effect of a strong magnetic field on the cooling efficiency[4]. Fortunately, the latter effects increase the cooling properties of the electron beam. Today the process of electron cooling is fairly well understood and has been studied extensively.

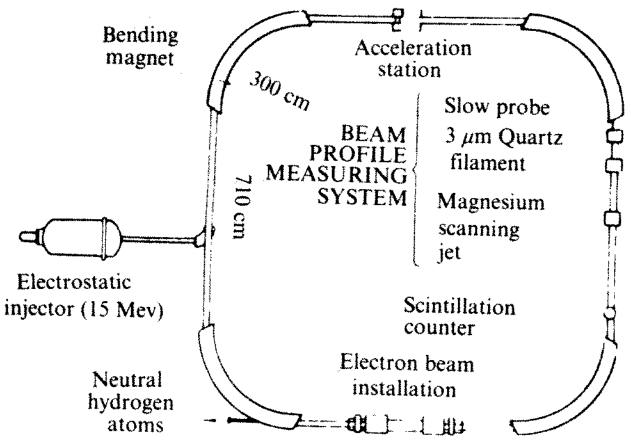


Fig. 1 Layout of the NAP-M proton accelerator.

The practical realisation of this technique started at the Nuclear Physics Institute in 1967. In 1974, the first experimental results on the electron cooling of protons were obtained, confirming that the fundamental ideas were correct (G. I. Budker, Ya. S Derbenev, N. S. Dukansky, I. N. Meshkov, V. V. Parkhomchuk, D. V. Pestrikov, R. A. Salimov, A. N. Skrinsky, B. N. Sukhina), while further studies of the technique by the same group indicated its high efficiency.

The experiments were carried out using a facility called the NAP-M (NAP is nakopitel antoprotonov—antiproton storage ring; M = model). The apparatus is shown in Fig. 1. Its perimeter is 47m and the length of each of the four straight sections is 7.2 m. The mean pressure of the residual gas in the vacuum chamber of NAP-M with the electron beam is $< 5 \times 10^{-10}$ torr. The stability of the magnetic field of the storage ring in the electron cooling regime is better than 1×10^{-5}. The control of the ring and the analysis of the experimental data was carried out by an on-line computer.

The electron beam for the cooling process comes from a source in the straight section of the storage ring (Fig. 2). The effective length of the cooling section is only 1/50 of the perimeter ($l = 1$ m, $\eta = 2 \times 10^{-2}$). An intense electron beam with low transverse velocities is obtained from a special electron gun, it is then guided in a longitudinal homogeneous magnetic field of 1 kG which extends up to the collector. The electron energy is recovered in the apparatus; this is especially important for high-energy electron-cooling devices.

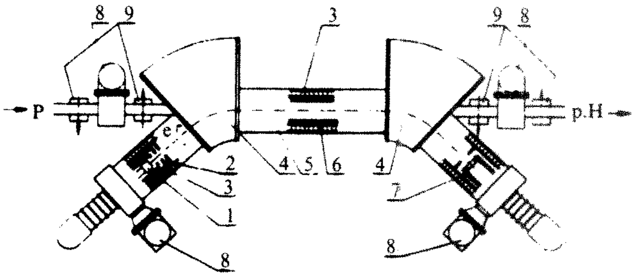


Fig. 2 Layout of an electron-beam installation: 1, electron gun; 2, anodes; 3, solenoid; 4, bending section of electron beam; 5, cooling section; 6, vacuum chamber; 7, collector; 8, vacuum pumps; 9, correction magnets.

The effect of electron cooling may be observed immediately, using a thin (0.5 mm) jet of magnesium vapour to measure the density of the proton beam. The jet intersects the proton beam, and the secondary electrons are collected by the electrostatic field onto a scintillation screen and recorded by a photomultiplier. When the jet scans the storage ring aperture, the transverse density distribution of the proton beam can be reproduced on an oscilloscope screen.

Without the electron beam, an increase in the dimension of the proton beam is observed due to multiple scattering on the residual gas. After the electron beam is switched in, the dimension of the proton beam decreases sharply. The effect of damping is seen especially clearly if the jet is kept at the centre of the damped proton beam and its dimension is increased by means of an inflector pulse. Then the density at the centre of the proton beam which is reduced after the pulse will increase to its equilibrium value within a characteristic time, τ.

The steady dimension of the proton beam is especially easy to measure with high accuracy by observing the beam of fast hydrogen atoms which is created in the cooling section as a result of the recombination of protons with the cooling electrons. It is interesting that even without special efforts more than 10% of the protons are converted into fast neutral atoms during the lifetime of the beam. At a distance of 10 m from the cooling section the dimension of the neutral beam is only 0.7 mm.

The damping of the energy spread of the proton beam is well demonstrated when the RF system of the storage ring is disconnected. If the energy of the electron beam is varied slightly, then the energy of the proton beam also varies and the radius of the proton orbit changes accordingly. If the energy of the electron beam and the magnetic field of the storage ring are varied simultaneously, then the proton beam can be accelerated or retarded without changing the equilibrium position of the orbit. Thus, for example, a proton beam was accelerated from 65 to 85 MeV during 200 s, corresponding to an energy gain by the protons of 0.3 eV per revolution.

Electron cooling was successfully verified in the proton energy range from 1.5 MeV (injection energy) to 85 MeV (0.4 GeV/c momentum) both for a freely circulating and for a bunched beam. The results obtained so far and the corresponding experimental conditions are given in Table 1.

The values of the cooling time τ and the strength of the longitudinal frictional force obtained in the experiment are better by almost two orders of magnitude than the values expected from the preliminary estimates[1]. It is now understood that cooling really depends on many different factors, the relative role of which is determined by the actual conditions in the storage ring with electron cooling.

Table 1 Typical experimental conditions and best results

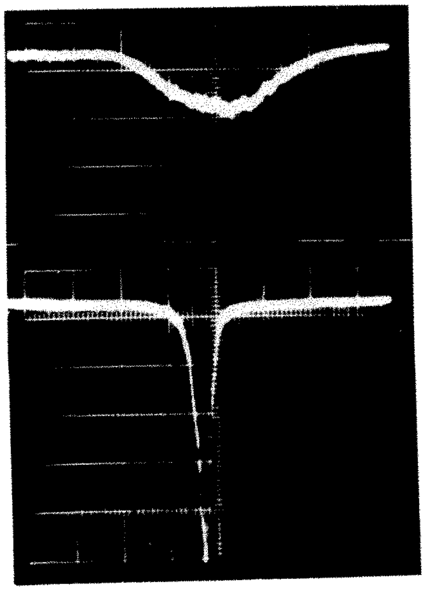
Proton momentum	0.35 GeV/*c*
Proton current	100 μA
Electron energy	35 keV
Electron current	0.5A
η	2×10^{-2}
Effective transverse electron temperature	0.2 eV
Effective longitudinal electron temperature	$<10^{-4}$ eV
Longitudinal magnetic field	1 kG
Cooling time	50 ms
Maximum longitudinal friction	1.5 MeV s^{-1}
Steady parameters of the cooled proton beam	
Diameter	0.5 mm
Angular spread	$\pm5\times10^{-5}$
Momentum spread	$<1\times10^{-5}$
Energy spread	<2 keV
Lifetime	10 h

Experimental possibilities

Electron cooling opens up possibilities in various areas of elementary particle and nuclear physics. Our survey[4] is devoted to their detailed discussion but here we shall confine ourselves to the most interesting examples which illustrate the promising nature of the electron cooling method.

Many experimental studies concern the storage of antiprotons, which is probably the most basic application of electron cooling. As an example, we shall give the parameters of the antiproton storage system proposed by the Nuclear Physics Institute for a planned accelerator storage ring complex at Serpukhov[4]. A simplified scheme for the complex is given in Fig. 4. Using the Serpukhov accelerator with the booster at present under construction (10^{13} protons per pulse, 2×10^{12} p s^{-1}), it is possible to accumulate 0.5×10^{6} $\bar{p}$ s^{-1} of 1.8 GeV/c momentum even without phase constriction of the proton beam in the accelerator. If we assume, optimistically, that it is possible to use the designed intensity of a proton beam of 10^{14} protons per pulse, then it is possible for a certain configuration of the system[4] to obtain 1×10^{9} $\bar{p}$ s^{-1}. But even an intensity of 0.5×10^{6} $\bar{p}$ s^{-1} would give a luminosity of up to 10^{31} cm^{-2} s^{-1} for $p\bar{p}$ intersecting beams of an energy of 2×10^{3} GeV. The first achievements in the storage of antiprotons have already allowed a wide range of new experiments to be carried out.

Fig. 3 Density distribution in proton beam before cooling (upper oscillogram) and after cooling (lower oscillogram). Horizontal scale: one large division corresponds to 1 mm.



In electron-cooled antiproton storage rings it is possible to perform a wide range of experiments with 'super-thin' internal gas and vapour targets. Here all the reaction products will be available for detection and analysis, including the slowly ionising fragments. In the superthin target case the antiproton energy losses and their fluctuation together with the multiple scattering of antiprotons on the target atoms are suppressed by the electron cooling, and the lift-time of the antiprotons is determined only by interactions with the target. For a good vacuum, the scattering on the residual gas is negligibly small. At high energies and for an accumulation rate of 10^{8} $\bar{p}$ s^{-1}, the luminosity may reach 10^{33} cm^{-2} s^{-1}, which is sufficient for a wide range of experiments.

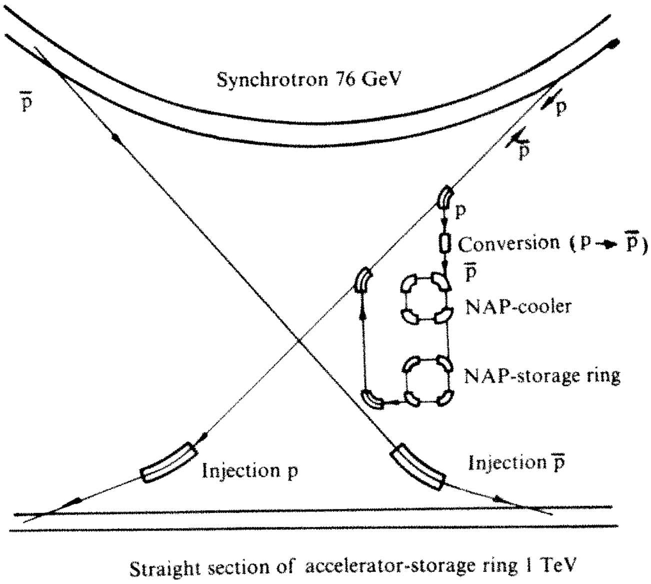


Fig. 4 Layout of proton–antiproton facility.

Superthin internal targets are particularly suitable for precision spectrometer experiments. Electron cooling in antiproton–proton and antiproton–nucleus experiments gives an energy resolution of the order of 10^{-4}–10^{-6} with satisfactory luminosity. Similar experiments with proton beams give the same high resolution, which is comparable with what can be attained in tandem accelerators and meson factories.

An important factor in spectrometer experiments is the possibility of very precise measurements of the absolute energy of particles of known mass in an accelerator by measuring the voltage accelerating the cooling electrons. Even today an accuracy of better than 10^{-5} may be attained.

Special possibilities are opening up in the study of $p\bar{p}$ interactions in experiments with continuously cooled beams in the energy range of 10 GeV per beam. Simultaneous cooling of the intersecting beams is possible even in a single ring (because the electrons, which cool protons, for example, will not perturb the antiproton beam, as its velocity has opposite direction). This set-up in comparison with the superthin target variant, may prove interesting if it is required to eliminate unwanted interactions with the target electrons and to reduce beam dispersion.

In particular, it is possible to investigate ψ-meson with a resolution much better than their intrinsic width, which is unattainable even in current experiments on e^-e^+ intersecting beams. Of course, only a very small proportion of all $p\bar{p}$ annihilation will take place via ψ-mesons even at resonance. Hence it is natural to look for the leptonic modes

$$p+\bar{p}\to\psi\to\begin{Bmatrix} e^{+}+e^{-} \\ \mu^{+}+\mu^{-} \end{Bmatrix}$$

The cross-sections of such processes at the resonance maximum is of the order of 100 nb. In a special storage ring with strong focusing at the intersection regions it is possible to obtain tens of thousands of such events per day.

We note that in $p\bar{p}$ collisions it is possible to obtain directly narrow states of the ψ-meson type with less strict constraints on quantum numbers than with e^-e^+ colliding beams, where the quantum numbers of such a state must be equal to those of the photon. It will be particularly easy to distinguish states with significant non-hadronic decay modes. An example of this type of meson is the $\chi(2750)$, decaying into two photons.

Using stored antiprotons, it is possible to obtain a beam of antihydrogen atoms. For this a parallel beam of cold positrons with the same mean velocity must be injected into one of the storage ring straight sections, through the electron-cooled antiproton beam. As a result of radiative recombination, the bulk of the antiprotons are converted into antihydrogen. Even on the NAP-M without special effort 10% of the protons are transformed into a beam of fast atoms.

Using this method it is possible to obtain beams of antiatoms of very low energy, which may permit neutral antihydrogen to be accumulated in a magnetic system which acts on antiatoms through the magnetic moment of the positron.

An important aspect of electron-cooled antiproton storage rings is the production of polarised beams of antiprotons. Today we consider the main variant to be the interaction of the stored antiproton beam with polarised superthin atomic hydrogen targets. According to existing data it is still difficult to choose optimal conditions combining a good degree of polarisation with minimal losses of antiptoton intensity.

One possible scheme is the interaction with longitudinally polarised protons in the storage ring section where the longitudinal polarisation of the antiprotons is stable. Here the difference in the total interaction cross-sections of antiprotons and protons of different relative helicities is used.

Polarisation experiments in storage rings seem feasible with antiproton beams. The method is adequate too for experiments with polarised protons and deuterons. Of course, polarised beams can only be obtained by directly storing polarised particles. The beams of accelerated polarised protons obtained so far and the existing polarised gas targets yield luminosities of not less than $10^{30}\,\mathrm{cm}^{-2}\,\mathrm{s}^{-1}$ under very clean conditions.

Electron cooling machines could already be proposed which may allow the intensity of the accelerated polarised proton beams to be increased to the level of existing proton beams of high-energy accelerators.

The use of electron cooling opens up the possibility of storing pure beams of antideuterons. The rate of accumulation of such beams is 10^{-4} of the rate of accumulation of antiprotons. In the superthin deuteron target case this would give luminosities of up to $10^{29}\,\mathrm{cm}^{-2}\,\mathrm{s}^{-1}$, which makes it possible to study in detail neutron–antineutron strong interactions, including annihilation processes. To distinguish these possibilities, it is necessary to register the antiprotons with small momentum loss and to select events with zero total charge of the remaining final particles, or to register the low-energy proton remaining after the decay of the neutron. The storage of antideuterons would be accompanied by the capture of mesons, antiprotons and antihyperons of momenta corresponding to the field and radius of the storage ring. It is not difficult to obtain a clean anti-deuteron beam: the mesons and antihyperons will decay rapidly, and the antiprotons may be removed by accelerating or retarding the antideuterons after storage with the aid of an electron beam, as described above.

There are also interesting prospects for experiments with stored ions, first in the superthin target regime using high monochromaticity of the cooled beams. Especially important is the use of electron cooling with ions which are difficult to obtain, principally heavy ions. However, working with these is complicated by radiative recombination with cooling electrons (the cooling is weakened in comparison with the radiative recombination proportionally to the mass of the ions). Hence it may be possible to cool these ions too, but it is impossible to keep them cooled for a long time. This problem may be solved by using proton cooling, for which the ion storage ring is supplemented by a proton storage ring, the straight section being common to both rings. The low temperature of the protons must be maintained by electron cooling.

Nevertheless, the most exciting of the immediate applications of electron cooling is still its use for experiments with intersecting $p\bar{p}$ beams at high energies.

By the time the first report on the principle of electron cooling appeared, a proposal for $p\bar{p}$ colliding beams (VAPP) had already been worked out in Novosibirsk. This proposal was published in 1971. It included a main storage ring with an energy of the colliding particles 2 × 23 GeV, the NAP antiproton storage ring of 1.1 GeV energy and an antiproton source. More recently a Fermilab project proposes to use the 500 GeV synchrotron as a $p\bar{p}$ colliding beam ring. Somewhat earlier the Nuclear Physics Institute had proposed the 2 × 2,000 GeV $p\bar{p}$ colliding beam project for UNK mentioned above. In this conservative approach a luminosity of $3\times10^{31}\,\mathrm{cm}^{-2}\,\mathrm{s}^{-1}$ may be attained, which is more than sufficient for carrying out first generation experiments.

The Fermilab project is in principle analogous to the VAPP/UNK project, except for one important point: the antiprotons 'generated' with an optimal energy of around 4 GeV after cooling are retarded to an energy close to 200 MeV which allows a certain gain in the storage rate. A synchrotron designed for periodic operation seems to allow intersecting beams of 2 × 300 GeV energy.

At CERN a project has been approved for a $p\bar{p}$ storage ring based on the 'stochastic cooling' method proposed in 1970 by Van der Meer.

Proton–antiproton ('quark–antiquark') intersecting beams permit the study of lepton pair creation processes.

$$p\bar{p} \rightarrow x \rightarrow \left\{ \begin{matrix} e^+ + e^- \\ \mu^+ + \mu^- \end{matrix} \right\}$$

in which both the electromagnetic and the weak interactions have a role. In particular, if the neutral intermediate boson Z^0 exists it may be found in these interactions if the total energy of the proton and antiproton exceeds the Z^0 rest mass by a factor of 3–6. Present estimates for its production require proton–antiproton beam energies in the region of 2 × (150–200) GeV. In this energy range, it would also be possible to observe the proposed charged vector basons $W^{\pm}$ of the weak interaction. It is also possible to search for heavy leptons in such experiments.

One can also study reactions in which a quark–antiquark pair is annihilated into a neutrino–antineutrino pair. Such a reaction is characterised by a visible energy reduced by a considerable fraction of the initial energy of the $p\bar{p}$ pair (of the order of 1/3); this loss will take place almost symmetrically with respect to the initial particles.

More detailed investigation will be possible with polarised $p\bar{p}$, and also pp and deuteron colliding beams. At the high energies considered, it is especially important to carry out experiments with longitudinally polarised particles at the intersection region. Electron cooling allows us to solve the problem of producing polarised proton and antiproton beams of the required intensity.

The quark approach to the interaction of protons and antiprotons emphasises the highly promising nature of experiments on colliding $p\bar{p}$ beams. But this does not exclude the possibility that the real physics of the interaction of matter and antimatter at super-high energies will prove to be still more interesting and entirely unexpected. In any case, there is no doubt that the first answer to these questions will be obtained in the next few years.

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articles

Bent beams and the overall size of extragalactic radio sources

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In a small sample of extragalactic radio sources, curvature is found to be a common feature in bright radio components coincident with quasi-stellar objects. In 3C273 and 3C345 the curvature is smooth between the nuclear (<0.003″) and the outer (>1″) regions. This contrasts sharply with objects having comparatively faint central radio components, which are often well aligned with the extended features.

ONE of the most significant facts to emerge from VLBI studies of extragalactic radio sources is the high degree of alignment between the most compact and the most extended features. The objects in which good alignment has been seen are 3C111, 3C236, 3C390.3, 3C405 and NGC6251 (refs 1–5). These are all 'classical double' radio sources with steep spectra and large overall size (>100 kpc). In contrast to this, it has been found[6,7] that on different scales some objects exhibit a range of position angles. It has become increasingly clear that, in these objects, the occurrence of non-aligned structure is strongly correlated with other important properties. This effect has been noted independently by Davis *et al.*[8].

Here we describe new observations which show that in some objects the position angle changes systematically with distance from the nucleus, through an angle ≥20°, that is the objects are curved. The observations enable us to rule out several possible explanations of the differing position angles. We discuss two models which are consistent with all the observations and which account naturally for some of the properties of these objects. These two models have widely different implications for the physics of extragalactic radio sources.

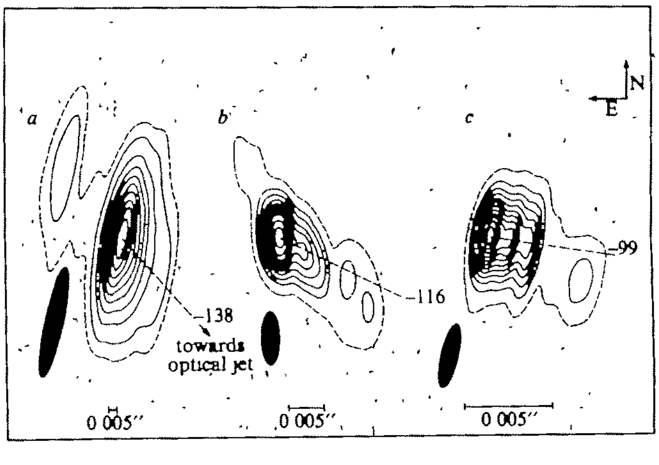
Straight and curved jets

Extragalactic radio sources can be divided into two major morphological groups: the first group consists of those objects in which the dominant radio emission comes from two components situated on opposite sides of the optical object and roughly equidistant from it. There may also be a radio component coincident with the optical object, but the total emission from it is small compared with that from the outer lobes. In general, these objects are large (≥100 kpc overall) and, at frequencies between ~200 MHz and ~5 GHz, they have steep spectra, $-1.0 \leq \alpha \leq -0.5$, where $S \propto \nu^{\alpha}$. We will refer to objects in this group as 'symmetric' or 'type S' objects, to emphasise that the dominant radio emission is roughly symmetrically placed on either side of the optical nucleus. The second group consists of objects in which there is a dominant compact (<1″) radio component coincident with the optical object, and if there are extended radio features these lie to one side of the optical object. These objects are generally small (≤100 kpc) and have flat spectra, $\alpha \gtrsim -0.5$, in the above frequency range. We will refer to objects in this group as 'core' or 'type C' objects, to emphasise that it is the coincidence of a dominant radio component with the optical nucleus which distinguishes them from type S objects.

Recent VLBI observations at 609, 1671, 5011 and 10,651 MHz not only confirm the different position angles seen on different scales in some objects, but also show that in at least two cases, 3C273 and 3C345, there is a smooth change in position angle between the compact and extended features. The details of the observations and analysis will be given elsewhere[9–11].

Figure 1 shows hybrid maps of 3C273 with three different resolutions made using the technique described by Readhead and Wilkinson[12]. The straight line through the brightest feature in each map, which we shall call the core, is drawn in the position angle of the feature closest to the core. Figure 1 shows that the

Fig. 1 Hybrid maps of 3C273 at three frequencies: *a*, 609; *b*, 5,011, *c* 10,651 MHz; showing the dominant structure on three different angular scales. The ellipses show the sizes (FWHM) of the restoring beams. The dashed line in each case is drawn through the radio core and the components closest to the core. The curvature of the source is clearly seen in both (*b*) and (*c*) maps. The systematic rotation of position angle from the smallest structure (*c*) to the largest structure (609 MHz map (*a*)) is also clear.



position angle of the radio structure in 3C273 rotates systematically with distance from the core. Figure 1*a* and *b* also shows features on the opposite side of the core, but further observations are needed to confirm these. The position angle of the larger-scale (~30 m arc s) structure agrees, to within the errors of observation, with that of the optical jet, which extends 20 arc s away from the nucleus. The total change in position angle, between the radio core and the optical jet, is 40°, over a distance of 70 kpc, assuming $H = 50\ \mathrm{km\ s^{-1}\ Mpc^{-1}}$ and $q_0 = 1/2$. Thus 3C273 is a type C object which shows pronounced curvature in the radio component coincident with the optical nucleus.

Four hybrid maps of 3C345 with different resolution are shown in Fig. 2. These again show a systematic change of position angle with changing resolution. In addition, Davis *et al.*[13] have discovered an elongated component in 3C345 which is 2 arc s away from the core in position angle (PA) −31°. This fits in well with the trend displayed by the source on smaller scales. Thus for 3C345 ΔPA = 45°.

In both 3C273 and 3C345 the curvature decreases as we move outwards from the core. Furthermore, the large-scale (>1 arc s) features in both cases are straight, that is they do not show curvature such as is seen near the core, and therefore, in both position and shape, these larger regions look like extensions of the compact inner regions shown in Figs 1 and 2.

These two sources provide the best evidence for a systematic rotation of position angle with distance from the radio core, but there are others which show the same tendency. For example, the 609-MHz hybrid map of 3C147 (ref. 6) shows a long narrow jet which is distinctly curved. This curvature is confirmed in a more recent map at 1,671 MHz (ref. 10). The large-scale (~0.7″) structure[14] also has a position angle different from that of the jet. The total change in position angle in 3C147 is 25°. Similarly, in 3C380 comparison of the 1,671 MHz hybrid map[10] with the Cambridge 5-km telescope map at 15 GHz (ref. 15) shows that ΔPA = 20°.

ΔPA, overall size, distance and morphology

Our observations of compact radio sources show that changes in position angle are common in type C sources, whereas as yet no example has been found of a non-aligned type S source. The data are given in Table 1 for nine sources, in order of increasing redshift. Figure 3 shows ΔPA plotted against the overall size, *D*, for each source. The sharp division in both ΔPA and *D* between core and symmetric objects is immediately obvious. These data show that the degree of alignment of the central radio objects is closely related to the overall size, the morphology and the distance class of extragalactic radio sources. The data shown in Table 1 should be viewed with some caution, however, as the sample of sources is not complete. But with the data presently available, the correlation between these four properties is very strong.

For this discussion we will assume that there is, in all cases, a unique source of high energy particles or radiation in the optical nucleus. We assume further that the energy is transferred to the outer components by a beam[16,17].

We have seen that type S objects tend to be large (>100 kpc) and to exhibit good alignment between compact and extended regions, while type C objects are curved and small (<100 kpc). The present observations are crucial, when seeking an explanation of these properties, because they enable us to eliminate some of the possible causes of non-alignment in type C objects. Rotation or precession of the energy source, or the possibility of two non-aligned beams, can be ruled out by the narrowness and alignment of the outer jets (3C273 and 3C345) or by the smooth curvature in 3C273 (Fig. 1). Similarly, curvature due to acceleration of the source by a nearby galaxy, as in 3C31 (ref. 18), would not explain why the curvature in 3C273 and 3C345 is greatest nearest the core. This would imply that the maximum acceleration, and hence the closest approach of the interacting galaxy, has occurred within the past few decades, which is very unlikely.

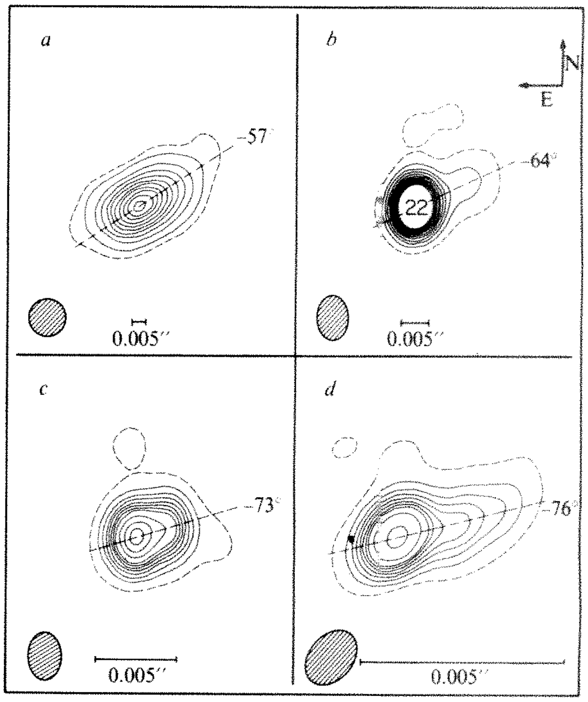


Fig. 2 Hybrid maps of 3C345 at four frequencies: *a*, 609; *b*, 1,667; *c*, 5,011; *d*, 10,651 MHz; showing the systematic rotation of position angle with structure on different scales.

Two possible explanations for the curvature are interesting because they can also explain the smaller overall sizes of type C objects.

(1) Beam bending by pressure: if the initial direction of the beam is not along the pressure gradient of the interstellar matter the beam could be bent by external pressure forces. The physical details of this mechanism have not been worked out, and the beam may be broken up by instabilities before bending through an appreciable angle. However, if it proves possible to bend the beam without destroying it, pressure bending becomes an attractive possibility because it could also explain many of the other observed features.

The bending of the beam by the external medium creates 'working surfaces' along curved portions of the beam. Thus we expect much greater interaction between the beam and the interstellar matter than in the case of a straight beam, which has only one working surface at the end. In sources bent by pressure we therefore expect to see 'hot spots' or knots along the beam, and we expect the brightest hot spots to coincide with the most strongly curved portions of the beam. This is entirely consistent

Table 1 Morphology, overall size, change in position angle and redshift of the objects in the sample*

Object	*Z*	*D* (kpc)	Type	ΔPA (deg)
NGC6251	0.023	2,900	S	≤4
3C111	0.0485	270	S	≤4
3C390.3	0.0561	320	S	≤5
3C405	0.0565	190	S	≤4
3C236	0.0989	5,700	S	≤3.5
3C273	0.158	70	C	40
3C147	0.545	5	C	25
3C345	0.594	25	C	45
3C380	0.691	40	C	20

Data either taken from text or from refs 1–5.
* Assuming $H = 50\ \mathrm{km\ s^{-1}\ Mpc^{-1}}$ and $q_0 = 1/2$.

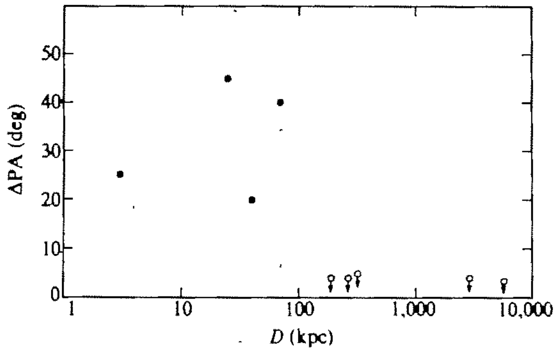


Fig. 3 The change in position angle (ΔPA) versus overall size (D) for the sources listed in Table 1: ○, type S sources, ●, type C sources.

with the observations of 3C273 and 3C345. Furthermore, much of the energy of a beam bent by external pressure forces would be dissipated inside the galaxy, and thus the beam would not travel as far. This could explain the smaller overall sizes of the curved sources and the bright synchrotron self-absorbed knots which we see in them[9]. In addition, on this model the curvature is greatest in the region of highest pressure gradient. We have seen, in 3C273 and 3C345, that the curvature is greatest at the core. This suggests the plausible explanation that the radio core is in the densest part of the galactic nucleus.

What causes the misalignment between the beam and the pressure gradient? This depends on the nature of the energy source. A strong possibility is a massive ($\gtrsim 10^8 M_\odot$) black hole[19–21], with the beam directed along the spin axis. As the black hole is formed from matter accreted from the galaxy, the angular momentum vector of the black hole, Ω_h, will be parallel to that of the nearby matter in the galaxy Ω_g. A strong gravitational interaction with another galaxy, which involves some transfer of matter, would change Ω_g to Ω'_g say, but leave Ω_h unaltered. Thus the angular momentum vector of the galaxy, and hence the pressure gradient, is no longer parallel to the beam. However, the black hole is accreting matter from the galaxy at a rate $\sim 1 M_\odot \text{yr}^{-1}$ (ref. 21), and after $\sim 10^7$ yr, by conservation of angular momentum, Ω_h will become aligned with Ω'_g. This scheme can also explain the good alignment observed in symmetric objects. We have seen that type S objects are larger, and therefore presumably older, than type C objects. Thus it may be that the good alignment in symmetric objects is related to their age. For example, Condon and Dressel[22] have shown that there is an increased probability of having a compact radio source in the nucleus of a galaxy if it is a member of a small group, or even a pair, of galaxies. Thus interaction with a nearby galaxy may trigger the production of a radio source as well as change Ω_g. This could explain why small (young) objects are curved whereas large (old, $>10^7$ yr) objects are well aligned.

(2) Projection effects: these must be present to some degree in every object and hence affect the values of ΔPA and D which we derive from the maps. They cause us to underestimate the total object size, and can lead to either an underestimation or an overestimation of ΔPA. They can give a natural explanation of both the curvature which we observe in type C objects and the alignment of type S objects, if we assume that all of these are formed by gently curving beams in which the total deviation from a straight line is very small ($\lesssim 5°$), and that in core objects the angle between the beam and the line of sight is small. A slightly curving beam appears strongly curved for most lines of sight almost parallel to the beam. If the radiating particles are moving at constant relativistic speeds along a narrow beam, the projection effects can also explain why the curvature is greatest nearest the core: the radiation is strongly beamed in the forward direction and the positions of maximum brightness and maximum curvature coincide as these both occur at the point where the beam is most nearly parallel to the line of sight. Therefore the majority of nuclear jets detected in a flux-limited sample will be directed towards the observer, and any intrinsic curvature will be strongly amplified by the projection effect. The one-sided structure in 3C273 and 3C345 shows that the radio core coincides with the origin of the beam. If this were not the case the maximum curvature and brightness would occur at an intermediate position along the visible portion of the beam.

The visible features in the maps all have roughly comparable flux densities, and, because the enhancement of apparent brightness by relativistic beaming is a very strong effect, this implies that all features in the maps must be moving at relativistic speeds towards the observer. If this were not the case, the intrinsic luminosities, over 4π sr, of the stationary and moving features would have to differ by large factors which fortuitously cancel the effect of relativistic beaming. Thus the intrinsic luminosity of the central radio sources in core objects is low, and they are not as different from the central sources in symmetric objects as they appear. In symmetric objects the beam is significantly offset from the line of sight which helps to explain why the type S objects in our sample are generally nearby whereas the type C objects are more distant, because we have to observe a large volume of space to find a significant number of objects with beams pointing towards us. Furthermore, the superluminal velocities[23], like those observed in 3C273 and 3C345, are expected on this model in sources in which the angle between the line of sight and the beam is small. This is true even if all the features are moving towards the observer at relativistic speeds. The relative motion which is observed could be due to the bending of the beam, as the apparent velocity depends on the angle between the line of sight and the beam, or it could be explained if the various features have differing velocities.

Projection effects alone cannot account for all of the observed differences between core and symmetric objects. This point is considered by Scheuer and Readhead[24], who show that some of the remaining differences can be removed by a simple extension of the model. The details of this model and the further implications for superluminal sources and for extragalactic objects in general are discussed elsewhere[24,25].

We suggest that one of the above possibilities, either projection of beam bending by the external medium, is the dominant effect responsible for the curvature in most type C objects. However, it is not possible to discriminate between these alternatives with the present observations. This may become possible in the future if we can follow the progress of individual radio knots along the beam. For example, if projection effects dominate, and if the radiating particles are moving with relativistic bulk velocity, v, along the beam, the apparent transverse velocity of a knot is a maximum at an angle θ to the beam, where $\cos\theta = v/c$. Thus, in a slightly curved beam the apparent transverse velocity passes through a maximum at the point where the angle between the line of sight and the beam is closest to θ. One might therefore see the knots speeding up as they approach this point and slowing down as they move away from it. Observations of such behaviour would be strong evidence in favour of the projection argument, although it might be possible to arrange conditions in the external medium to mimic the effect in a beam bent by pressure forces. Conversely, if the velocity of the knots is found to be constant over a substantially bent portion of the beam this would be strong evidence against the projection argument.

Conclusion

We have shown that the occurrence of non-aligned radio structure in the nuclei of extragalactic radio sources is strongly correlated with three other important properties—morphology, overall size and redshift. Our observations have enabled us to rule out several possible explanations for the misalignment of type C objects. Two plausible explanations consistent with the observations are external pressure bending and projection. These are totally different in many important physical aspects. If the curvature is due to external pressure forces then the bulk velocity of the radiating particles along the jet could be nonrelativistic, and the jet one-sided, that is we see all of the object.

If we are observing a projection effect in a jet with relativistic bulk motion, the jet could actually be two-sided, that is we may only see half of the object. The projection effect can only amplify a pre-existing curvature, however, and pressure may be responsible for this curvature.

Both models can explain the coincidence of maximum curvature with maximum brightness, but the projection argument is more attractive because it also accounts automatically for the superluminal velocities observed in some type C objects and the correlation of curvature with distance.

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Olivine fractionation equations for basaltic and ultrabasic liquids

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Exact equations are derived for fractionation curves of basaltic and ultrabasic liquids. The ideal fractionation equation has the form $y = ax^K$ *while equilibrium crystallisation has the form* $(1/y) = Km_1(1/x) + b_1 \cdot (1-K)$, *and batch fractionation,* $y = Km_2 + b_2 \cdot (1-K)$. *A ratio transformation as in Pearce diagrams allows natural data to be analysed to determine if a constant distribution coefficient fits the data: K may also be determined directly. Published data for a series of liquids fractionated from a lunar picrite parent have been analysed using the equilibrium equation. The average K value is* $K = 0.323$ *with a linear correlation coefficient,* $R = 0.996$. *The reported olivine analyses yield a* $K = 0.321$ *for the same set of experiments. Using the ideal fractionation equation, the liquid line of descent can be calculated and constraints placed on the primitive liquids which produced a given mafic rock by olivine fractionation.*

GIVEN the chemical composition of a series of rocks allegedly representing liquids which have fractionated olivine from a single parent, we should be able to use fractionation curves of the type introduced by Bowen[1], and Osborne and Schairer[2] to test this hypothesis. However, until the present work, the equations of exact fractionation curves requiring only chemical data had not been developed. This problem may be treated by rather complicated equations involving the fraction of material transferred from liquid to solid (see ref. 3). Of course, in dealing with natural data, the fraction of liquid converted to solid is not generally known and must be estimated. These equations, although complex, may be used to generate models on a trial and error basis. Alternatively, a step-wise computer program such as developed by Roeder[4] can approximate the fractionation curve (however, the increments must be small because the error is cumulative).

Unless the equations are known explicitly, it is difficult to judge how well the natural data fit various fractionation models, and extremely difficult, if not impossible, to derive a model directly from the data.

Ideally one would like to express the fractionation, either ideal (Rayleigh-type) or equilibrium, in such a simple form that it can be rearranged in a linear form with predictable slope and intercept. The direct analysis of observed distribution coefficients would then be possible and trial and error methods would be unnecessary.

Here, I will develop such equations in absolute units and show how they may be used to analyse natural data to determine not only if the distribution coefficient is constant, but also to evaluate it directly from the natural data. To use the equations, the data must be transformed using a simple transformation[5,6] which eliminates the closed array property of data expressed as a per cent (or fraction of the whole). Many petrologists may not be familiar with the properties of such a transformation, and I will briefly explain the technique below.

Some theoretical considerations

In 1968, I proposed that certain types of ratio diagrams in which the denominator is a constant (Pearce diagrams) be used to analyse data in which a system may be considered to have progressively changed composition. The actual chemical rate of change of constituents in such a case is modelled exactly by considering absolute amounts of the constituents (grams, tons, mols and so on). In so doing, we are treating chemical variables exactly like the extensive (thermodynamic) properties of thermodynamics.

Erroneous results may be obtained by considering variations in weight per cent. Harker diagrams, for example, are particularly misleading as they plot SiO wt % on the abscissa as though it were an independent variable. SiO_2 wt % is not a single variable representing the silica content of a rock, it is rather a complex variable composed of at least 11 other variables (the other components of the rock). The closed array or, constant sum property, of weight per cent data also causes difficulties for statistical manipulations[7].

There is another problem with per cent data (and all natural chemical data). That is, the variables cannot be separated to give the absolute units which exactly describe the system. It is extremely difficult to apply the usual mathematical techniques to a complex variable (such as wt % SiO_2), itself composed of 12 simple variables. A ratio transformation of the data produces a mathematically simpler form (we only have two variables). The simplest possible result occurs when the denominator is a constant as in Pearce diagrams. It is a characteristic of this transformation that it allows us to obtain the same results as though the variables were separated.

This method of treating data is explained as follows, if we have a relationship between y and x, say

$$y = f(x) \tag{1}$$

then

$$\left(\frac{y}{z}\right) = g\left(\frac{x}{z}\right) \tag{2}$$

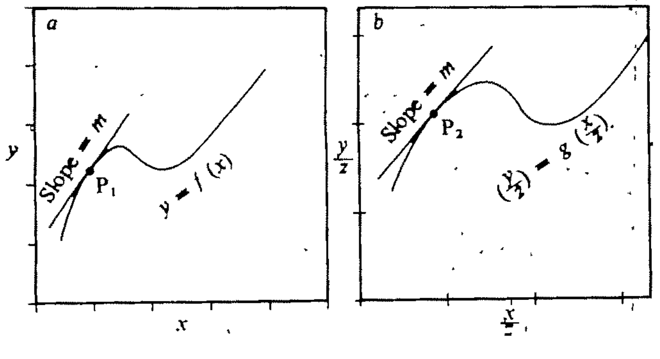
Equation (1) has the same form as (y/z) versus (x/z), from equation (2), only if z is a constant in the mathematical sense. As $y/z = y\%/z\%$, these ratios are easily evaluated. In effect we are magnifying the curve $y = f(x)$ without changing its shape. Examining the problem graphically, we wish to obtain the relationship in Fig. 1*a*. We cannot do this because we cannot obtain the absolute values of y and x from closed array data (percentage values). We can, however, obtain the graph in Fig. 1*b* from which we have the same important information as from Fig. 1*a*, namely the rate of change of y with respect to x.

Every point on $y = f(x)$ has a corresponding point on $(y/z) = g(x/z)$ which bears the same relationship to y and x (that is, the slopes are the same). Thus, we can determine the true relationship between y and x by plotting $y\%/z\%$ against $x\%/z\%$ remembering that z must be a constant. This is the point of the transformation of data in a Pearce diagram and has practical applicability far beyond its mathematical simplicity. I shall use it here to calculate the distribution coefficient between olivine and liquid using only the composition of a series of liquids from which olivine has been fractionated.

Ideal fractionation equations

During ideal fractional crystallisation it is assumed that the crystals are effectively removed from the liquid as they are formed so that there is no reaction between crystals and liquid. In this case, we know, therefore, that the chemical rate of change of the liquid composition is the same as the composition of the olivine being precipitated. If we assume the distribution coefficient is constant, we can now derive the equation for ideal fractionation of olivine from the liquid as follows.

Fig. 1 *a*, An hypothetical graph of two related variables y and x in absolute values (grams, tons, mols) In general this type of graph cannot be determined from natural chemical data; *b*, as for *a* but with the data transformed by dividing by z, a constant. Point P_2 corresponds to point P_1 and has the same slope. In fact, as corresponding points yield the same slopes, the curve has the same shape as in (*a*).



Let y = amount of FeO in absolute units (such as, grams, tons, mols); x = amount of MgO in the same units as y; K = distribution coefficient as defined by Roeder and Emslie[8].

$$\left(\frac{\mathrm{FeO}}{\mathrm{MgO}}\right)_s = K\left(\frac{\mathrm{FeO}}{\mathrm{MgO}}\right)_L \tag{3}$$

where subscripts S, refers to solid or crystals; L, refers to liquid; i, refers to the 'i' differentiate formed; p refers to the parent. x_0 = the original amount of MgO in the system (parental MgO); y_0 = the original amount of FeO in the system (parental FeO); c_1 and so on = a constant.

Now

$$\left(\frac{y}{x}\right)_s = K\left(\frac{y}{x}\right)_L \tag{4}$$

As the crystals are physically removed from the liquid the instant they form, it follows that

$$\left(\frac{dy}{dx}\right)_L = \left(\frac{y}{x}\right)_s \tag{5}$$

considering the liquid path

$$\left(\frac{dy}{dx}\right)_L = K\left(\frac{y}{x}\right)_L \tag{6}$$

or

$$\frac{dy}{y} = K \cdot \frac{dx}{x} \tag{7}$$

integrating equation (4)

$$\int \frac{dy}{y} = K \int \frac{dx}{x} + C_1 \tag{8}$$

$$\log y = K \log x + C_1 \tag{9}$$

evaluating the integration constant C_1

$$\log y = K \log x + \log (y_0/x_0^K) \tag{10}$$

or

$$y = \left(\frac{y_0}{x_0^K}\right) \cdot x^K \tag{11}$$

These simple equations describe the exact shape of the ideal fractionation curve in absolute amounts and may be used to model olivine fractionation. They cannot be used to analyse natural data because natural chemical data cannot be separated into discrete, simple, variables. However, by using the transformation of a Pearce diagram, we can achieve the same result. Therefore, using theorem 1 of Pearce[5]: y/z versus x/z will give us the same relationship as y versus x, and therefore

$$\log\left(\frac{y}{z}\right) = K \log\left(\frac{x}{z}\right) + \log\left(\frac{y_0}{x_0} \cdot z^{K-1}\right) \tag{12}$$

and

$$\left(\frac{y}{z}\right) = \left(\frac{y_0}{x_0}\right) \cdot \left(\frac{z_0}{x_0}\right)^{K-1} \cdot \left(\frac{x}{z}\right)^K \tag{13}$$

Both equations (12) and (13) may be used to analyse natural data but equation (12) is more useful as it gives a straight line with slope K on a $\log(y/z)$ versus $\log(x/z)$ diagram. Thus the presence of a constant distribution coefficient as well as its value may be determined from natural liquid compositions. Rewriting equation (12) in terms of oxide constituents

$$\log\left(\frac{\mathrm{FeO}}{\mathrm{Al_2O_3}}\right) = K \log\left(\frac{\mathrm{MgO}}{\mathrm{Al_2O_3}}\right) + \log\left(\frac{\mathrm{FeO}}{\mathrm{MgO}}\right)_p \cdot \left(\frac{\mathrm{Al_2O_3}}{\mathrm{MgO}}\right)_p^{K-1} \tag{14}$$

Note that in equations (12) and (13) the exact size of the system need not be specified as the equation does not change with the size of the system.

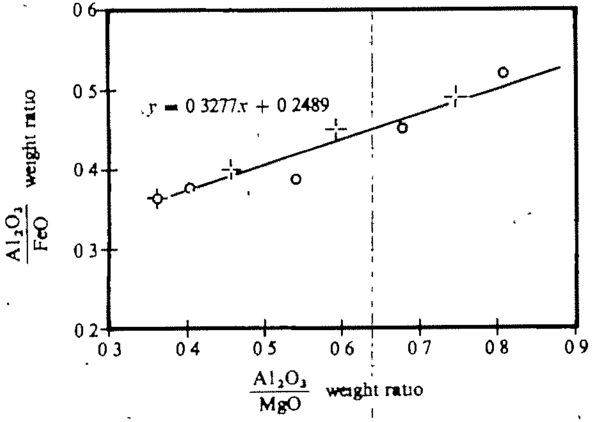


Fig. 2 Weight ratio data for fractionated liquids from a lunar picrite. The points are both actual experimental data (O) and smoothed data (+). The linear regressive line has a linear correlation coefficient of $R = 0.996$. The average K determined from both slope and intercept is 0.323. Walker *et al.* report data giving an average K of 0.321 Note that this type of 'inverted data' graph should only be used to analyse the K values for liquids which have formed by equilibrium crystallisation.

Equilibrium crystallisation equations

During equilibrium crystallisation there is continuous, complete reaction between the crystals and coexisting liquid. At any instant there is only one solid composition which represents the total amount of crystals which have formed, and the system consists of only two phases, crystals and liquid. Unlike ideal (non-reactive) fractionation, we do not know, at first sight, the chemical rate of change of the liquid (dy/dx), however, we do know that, because of the constant composition constraint, (in absolute amounts):

$$y_0 = y_s + y_L \tag{15}$$

and

$$x_0 = x_s + x_L \tag{16}$$

also

$$\left(\frac{y}{x}\right)_s = K\left(\frac{y}{x}\right)_L \tag{4}$$

therefore, combining equations (15), (16) and (4), in terms of the liquid curve

$$\left(\frac{y}{x}\right)_L = \frac{1}{K}\left(\frac{y_0 - y_L}{x_0 - x_L}\right) \tag{17}$$

As we are concerned only with the liquid, we can drop the subscripts and rearrange equation (17) by cross multiplication to give the simple form

$$\left(\frac{1}{y}\right) = K\left(\frac{x_0}{y_0}\right)\left(\frac{1}{x}\right) + \frac{(1-K)}{y_0} \tag{18}$$

This is the equation of a straight line with slope $K(x_0/y_0)$ and y-intercept $(1-K)/y_0$ if $(1/y)$ is plotted against $(1/x)$. This equation may be used for modelling but it cannot be used directly to analyse natural data because it is written in absolute amounts. Using the transformation advocated here, substituting y/z and x/z for y and x

$$\left(\frac{z}{y}\right) = K\left(\frac{x_0}{y_0}\right)\left(\frac{z}{x}\right) + \left(\frac{z_0}{y_0}\right) \cdot (1-K) \tag{19}$$

Rewriting in terms of oxide constituents

$$\left(\frac{Al_2O_3}{FeO}\right)_l = K\left(\frac{MgO}{FeO}\right)_p \cdot \left(\frac{Al_2O_3}{MgO}\right)_l + \left(\frac{Al_2O_3}{FeO}\right)_p \cdot (1-K) \tag{20}$$

These ratios, which are readily determined from chemical analyses, may be in any units and K calculated from the slope will be the same. Of course, any element, oxide or extensive variable of the system could be used as the denominator of the ratios as long as it is constant. However, it is convenient to use Al_2O_3 or $(Al_2O_3 + CaO)$, although TiO_2 is also useful (especially if one attempts to model plagioclase fractionation in addition to olivine).

Batch fractionation equations

In batch fractionation, which is essentially a disequilibrium non-reaction process, we assume a constant composition of olivine determined by the original liquid composition and distribution coefficient. The equation for such a process is

$$y = K\left(\frac{y_0}{x_0}\right)x + y_0 \cdot (1-K) \tag{21}$$

or applying the transformation as in Pearce[5]

$$\left(\frac{y}{z}\right) = K\left(\frac{y_0}{x_0}\right)\left(\frac{x}{z}\right) + \left(\frac{y_0}{z_0}\right) \cdot (1-K) \tag{22}$$

Rewriting in oxide form using Al_2O_3 as a divisor we have

$$\left(\frac{FeO}{Al_2O_3}\right)_l = K\left(\frac{FeO}{MgO}\right)_P \cdot \left(\frac{MgO}{Al_2O_3}\right)_l + \left(\frac{FeO}{Al_2O_3}\right)_P \cdot (1-K) \tag{23}$$

Analysis of liquid composition

If we have the compositions of a series of liquids produced by ideal fractionation of olivine, then from equation (14) it is clear that a plot of (FeO/Al_2O_3) versus (MgO/Al_2O_3) will be linear with a slope of $m = K$ and a y-intercept of

$$b = \log\left(\frac{FeO}{MgO}\right)_P \cdot \left(\frac{Al_2O_3}{MgO}\right)_P^{K-1} \tag{24}$$

Thus we have two ways of evaluating K from a single graph.

Unfortunately I do not have access to natural data to demonstrate the effectiveness of this technique. However, data are available for equilibrium crystallisation and that will be analysed below.

Table 1 Lunar picrite observed and calculated liquid compositions

	1	2	3	4
	180	184		1250
FLIQ	1.00	0.83	0.83	0.84
Olivine mol %	77.3	69.4	68.7	72.1
Olivine observed	—	69.7	—	—
SiO_2	43.8	44.0	45.3	44.3
TiO_2	2.80	3.33	3.4	3.3
Al_2O_3	7.95	9.63	9.68	9.5
FeO	22.0	21.3	21.7	21.1
MnO	0.29	—	0.35	—
MgO	13.5	8.69	8.55	9.8
CaO	8.18	9.95	9.96	9.5
Na_2O	0.22	—	0.27	0.30
K_2O	—	—	0	0.07
Cr_2O_3	0.65	—	0.79	1.0
	99.39	96.90	100.00	98.87

Analyses 1, 2 and 4 from Walker *et al.*[9]. FLIQ is the fraction of liquid remaining after fractionation. Olivine mol % is the equilibrium olivine calculated from equation (3) assuming a K of 0.32. Olivine observed is the actual olivine analysed by Walker *et al.*[9]. 1. Starting composition, lunar picrite 12002. 2. Liquid fractionated from 12002 by olivine fractionation. 83% of the liquid remains. Compare this composition with nos 3 and 4. 3. Fractionated liquid calculated using equation (18) and summing the absolute amounts to 100%. Note the close agreement with the experimental run no. 2. 4. Walker *et al.*[9] reported this fractionated composition for 1,250 °C and 84% liquid remaining. Compare with nos 2 and 3 Note that the MgO content is slightly high resulting in a calculated olivine slightly enriched in forsterite.

In the case of equilibrium crystallisation it is clear from equations (19) and (20) that a plot of (Al_2O_3/FeO) against $(Al_2O_3)/MgO) \cdot (MgO/FeO)_p$ will be linear with a slope of $m = K$, and a y-intercept of

$$b = \left(\frac{Al_2O_3}{FeO}\right)_p \cdot (1-K) \qquad (25)$$

Thus, in this case also, we have two different ways of evaluating K from the same graph.

Walker *et al.*[9] have reported experimental data for equilibrium crystallisation of olivine from a lunar picrite. These data are plotted in Fig. 3 and may be used to evaluate the nature and value of the distribution coefficient. Note that in this case the x-values are multiplied by $(MgO/FeO)_p$ to yield a slope of K. Using Walker's fractionated liquid values the observed K calculated from the liquids is 0.328 (from the slope) and 0.319 (from the intercept) giving an average value of 0.323. This compares favourably with the average K of 0.321 calculated from the reported liquid and olivine compositions. Table 1, shows compositions calculated using equation (18) and summing the absolute amounts to 100%: the excellent agreement requires no further comment (see Fig. 3).

Table 2 Calculated parental liquids for an abyssal tholeiite

	1	2	3	4	5	6
FLIQ	1.000	1.183	1 355	1.521	1 685	1.846
Olivine mol%	82.2	88 2	90 9	92 42	93 4	94.2
SiO_2	50.00	48.51	47 57	46.90	46 39	45.99
TiO_2	1 64	1.39	1 21	1.08	0 97	0.89
Al_2O_3	15 00	12.68	11 07	9.86	8.91	8.13
Fe_2O_3	0.40	0 34	0 30	0 26	0 24	0 22
FeO	10 80	11.24	11 09	10.76	10 39	10 02
MnO	0 22	0.19	0.16	0.15	0 13	0 12
MgO	8.40	14.20	18 61	22.09	24 93	27.31
CaO	10 60	8 96	7.83	6.97	6 29	5 74
Na_2O	2.70	2.28	1.99	1.78	1.60	1.46
K_2O	0.13	0 11	0 10	0.09	0.08	0.07
P_2O_5	0.11	0 09	0 08	0 07	0.06	0 06
H_2O	0.00	0.00	0.00	0.00	0 00	0.00

1, Abyssal tholeiite studied by Fujii and Kushiro[10] The composition was adjusted to give $Fe_2O_3 = 0\ 40$ and summed to 100% 2–6, Possible parental liquids for no. 1 calculated using equation (11) and summing the absolute amounts to 100%. The assumptions are: $K = 0.30$ and ideal fractionation of olivine Composition no. 4 has 52 1% olivine added and is in equilibrium with olivine of $Fo_{92.4}$. Composition nos 1–6 define the olivine-controlled liquid line of descent which leads to the abyssal tholeiite no 1

Calculation of primitive liquids

There is another way in which the explicit equations developed here may be used in petrogenetic theory. The family of curves described by equation (11) are, in effect, olivine-controlled liquid lines of descent. Considering the form of the equations, it is clear that a single point on the curve defines the equation. Therefore, only one ideal fractionation curve passes through any given composition. The consequence of this is important. If a single differentiate is known, then all possible parental liquids may be calculated exactly. As ideal fractionation is a limiting case, limits may be placed on the compositions of peridotitic liquids which may be parental to a given basic or ultrabasic liquid; always assuming, of course, that K is constant, and known. Table 2 shows a set of primitive liquids calculated for a recently studied abyssal tholeiite[10].

Unlike ideal fractionation, the equation for the family of equilibrium curves contains two parameters (in addition to K). Thus two points on any curve are necessary to define that curve. As a consequence of this, a given mafic liquid never lies on a single equilibrium crystallisation line of descent. An infinite number of equilibrium curves pass through any given composition. Thus the constraints on possible parental compositions are more complicated than in the case of ideal fractionation. A discussion of the details of determining the parent line of composition is beyond the scope of this work, and will be reported later.

Discussion

While developing these equations, some problems of applicability to natural data became apparent. First, most petrologists are still publishing whole rock analyses of porphyritic rocks. Bowen[11] has pointed out that whole rock compositions do not necessarily represent liquids, hence whole rock compositions have limited value in petrogenetic studies. The modern approach would seem to be to analyse the matrix separately. Such data would give the liquid line of descent directly and be more useful in theoretical studies. Attempts to correct whole rock analyses to a phenocryst-free composition by using modal analyses are unsatisfactory. The errors introduced by the modal analyses are such as to render the resulting data virtually worthless. Second, the value of K determined from the above equations is extremely susceptible to errors in analyses. Therefore, it is suggested that in some studies replication of analyses may be useful. Alternately, smoothing the data to a curve, as in ref. 9, seems to work well, especially if there are only a few points on the line.

Although calculation of liquid composition is the main concern of the present work, calculation of the total solid composition is readily accomplished by subtracting the liquid composition at any point from the starting composition.

In spite of the different form of the equations for ideal and equilibrium fractionation, the results for differentiation of basalt are rather similar. In fact, for small degrees of fractionation they may be difficult to distinguish. However, the two equations are fundamentally different. If, for example, at any point during equilibrium crystallisation the liquid is isolated from the solid, then the system initiates a new curve from that point. For ideal fractionation, on the other hand, only a single curve exists.

It may be worth noting that the fractionation curves are independent of temperature as long as K is independent of temperature. The equations reported here, although developed for olivine fractionation, are completely general, and were developed in terms of y and x to emphasise this generality. The equations, therefore, apply not just to olivine, but to any mineral for which K is constant and which has a solid solution between two end-members. The equations apply directly to orthopyroxene and clinopyroxene (if conditions are such that K is constant). Plagioclase is a more difficult case, but similar equations are being developed for plagioclase fractionation and these will be published later.

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Degenerate perturbations of protein structure as the mechanism of anaesthetic action

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The interaction of the n*-alkanols with lipid bilayers and excitable membranes shows that there is no simple correlation between conduction block and any of the perturbations of bilayer structure currently proposed as unitary mechanisms of local anaesthetic action. We propose instead that the* n*-alkanols act by direct interaction with target proteins to cause perturbations which depend directly on the precise structure of the alcohol.*

A CLASSICAL EXAMPLE of nonspecific drug action lies in the effects of local anaesthetics on isolated nerve preparations. The extraordinary range of chemically diverse structures which will cause reversible conduction block suggests that there can be no specific chemical interaction between a unique receptor site on the proteins responsible for nerve conduction and all the molecules which have local anaesthetic activity. Nevertheless, it has been widely assumed as a working hypothesis that there may be a common structural perturbation in the excitable membrane which is caused by all anaesthetics irrespective of their structure and which is sensed by the target proteins[1]. The underlying rationale for such unitary hypotheses derives from the experimental evidence that anaesthetic potency correlates approximately with the oil–water partition coefficient of the anaesthetics[2], implying that anaesthetic action occurs when about the same number of molecules interact with the excitable membrane. The assumption is that, as the bound anaesthetics at the same membrane concentration all perturb function to about the same extent, they may all do so by the same molecular mechanism[3].

However, there has been no direct experimental evidence for any unitary mechanism because it has not yet been possible to analyse directly the interaction of anaesthetics with the target protein structures in the excitable membrane. For this reason many other membranes[4,5] have been used as models to analyse the perturbation of membrane structure and function by anaesthetic molecules. Unfortunately, as a direct consequence of the nonspecific nature of the interactions, virtually every physical and biochemical parameter which can be measured may be affected, many at, or just above, the concentrations required for local anaesthetic action[6]. Despite the problem of identifying the relative importance of these many perturbations, studies have focused attention on two contrasting classes of structural perturbation as candidates for a unitary mechanism of anaesthetic action. These are perturbations of the structure of the lipid bilayer leading indirectly to perturbations of the target protein structures, or alternatively, perturbations of the target protein structures through their direct interaction with the anaesthetics.

Here we compare the effects of n-alkanols on various properties of the lipid bilayer with their ability to block nerve conduction, which provides a critical test to distinguish between these two main hypotheses.

Perturbations of fluidity and phase transitions by *n*-alkanols

The effects of the n-alkanols on the fluidity of a range of defined lipid bilayers was estimated using the spin label technique. The ratio of the low-field to mid-field peak heights (h^{-1}/h^{0}) was used as an arbitrary measure of bilayer fluidity to demonstrate the effects of fluidity on the short- and long-chain n-alkanols. As an example, Fig. 1 (see legend for details) shows the effects of butanol and dodecanol, separately and in combination, on dioleoyl phosphatidylcholine (DOPC) and oleoyl palmitoyl phosphatidylcholine (OPPC) with and without cholesterol, for spin-labelled phospholipids carrying the nitroxide group at C-16. These lipid mixtures were selected to span the range of bilayer fluidity likely to be found in excitable membranes. Similar results were obtained when the spin label was in the C-7 or C-10 position (not shown).

In general, a similar pattern of opposing perturbations and antagonistic effects of the two n-alkanols in combination is obtained irrespective of the composition of the lipid bilayer or the spin label used. In detail, the magnitude of the effects varies, in that the decrease in fluidity caused by dodecanol is small in bilayers containing high proportions of cholesterol, which is itself a potent rigidifying agent, and the spectral changes observed with the nitroxide at the C-7 position are marginal with either alkanol. Similar results are obtained with other pairs of short- and long-chain n-alkanols; n-alkanols with a chain length of <10 carbon atoms increase membrane fluidity whereas those with a chain length >10 carbon atoms decrease it (not shown). The general conclusion, that the short- and long-chain n-alkanols cause opposing changes in bilayer fluidity holds not only for the defined lipid mixtures, but also for bilayers of lipids extracted from erythrocytes (not shown).

We assume that the same conclusion will probably apply to the effects of the alcohols on the fluidity of the lipid bilayer in excitable membranes, particularly as the lipid composition of the axonal membranes closely resembles that of the erythrocyte membrane[7,8]. A similar pattern of responses emerges from the effects of the n-alkanols on the transition temperature ($T_t = 23.5$ °C) of dimyristoyl phosphatidylcholine (DMPC) (Fig. 2*a*); the short-chain alkanols depress T_t, whereas the long-chain alkanols increase T_t, as reported previously[9,10]. The effect of butanol on DMPC–dodecanol mixtures (2 : 1 and 1 : 1) shows the consistent antagonistic action of the combined alkanols on T_t (Fig. 2*b*). Similar results were obtained with dipalmitoyl phosphatidylcholine, and the increase in T_t with the long-chain alkanols does not therefore depend on any precise relationship between the chain length of the alkanol and that of the lipid.

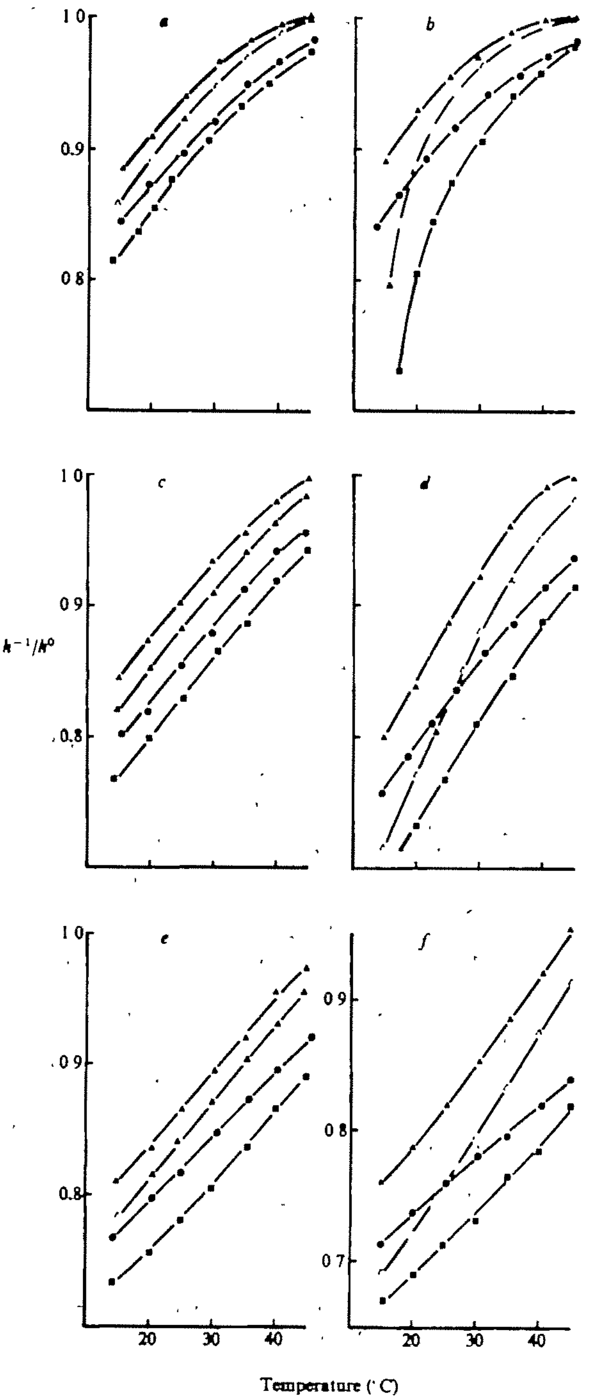

Fig. 1 The effects of butanol (C_4) and dodecanol (C_{12}) on the fluidity of dioleoyl phosphatidylcholine (DOPC) and palmitoyl phosphatidylcholine (OPPC) bilayers, and mixtures of these lipids with cholesterol (Ch). The phospholipids, synthesised by the method of Robles and van den Berg[36], were dissolved in $CHCl_3$ with spin label (1 mol%), with or without added cholesterol and C_{12}. The samples were dried under N_2 and resuspended in 100 mM potassium phosphate buffer, pH 7.2, to give a final concentration of 75 mg ml^{-1} phospholipid. For experiments in 150 mM C_4, the alkanol was added to the buffer before resuspension of the dried lipid samples. The spin labels used were the N-oxyl-4',4'-dimethyloxazolidine derivatives of 1-palmitoyl-2(n-ketostearoyl) phosphatidylcholine ($n = 7$, 10 or 16) (results for $n = 7$, 10 not shown). Spectra were recorded on a Varian E3 EPR spectrometer with a variable temperature controller. The fluidity was estimated arbitrarily from the ratio of the low-field to mid-field peak amplitudes (h^{-1}/h^0) (ref. 37), where an increase in this ratio indicates an increase in bilayer fluidity. *a*, DOPC; *b*, OPPC; *c*, DOPC/Ch, 4/1; *d*, OPPC/Ch, 4/1; *e*, DOPC/Ch, 2/1; *f*, OPPC/Ch, 2/1. ▲, C_4 (150 mM); △, C_4+C_{12}; ●, no alkanol; ■, C_{12} (2 lipid · 1 alkanol).

Nerve-blocking action of alkanols

The local anaesthetic actions of the primary *n*-alkanols were investigated using an *in vitro* preparation of the lateral olfactory tract (LOT) of the guinea pig. This nerve consists of thinly myelinated nerve fibres of uniform size (1–2 μm diameter) which conduct impulses at 6–8 m s^{-1} (refs 11, 12). We chose this for our experiments as it does not possess the layers of connective tissue characteristic of peripheral nerves that may present a barrier to the diffusion of the less soluble alkanols.

A well defined biphasic compound action potential of 1–6 mV amplitude and 1.5 ms duration was recorded along the length of the nerve after a supramaximal stimulus to the anterior end of the LOT[13]. On exposure to butanol or octanol the first indication of the start of nerve block was an increase in both the latency and duration of the compound action potential. Thereafter, the amplitude of the action potential declined progressively and the latency and duration increased still further, until the nerve was completely blocked. For concentrations of butanol and octanol just sufficient to block nerve conduction this sequence of events took about 15 min (Fig. 3). The actions of the long-chain *n*-alkanols (C_{10}–C_{12}) were similar to those of butanol and octanol. The time taken to achieve nerve block increased progressively as the water solubility of the *n*-alkanols diminished, being up to 700 min for dodecanol (Fig. 3). In the absence of anaesthetic the fibres of the LOT conducted normally for periods in excess of 12 h. Conduction block by all the *n*-alkanols up to C_{11} was shown to be reversible. The effect of dodecanol could only be partially reversed even when nerve block was incomplete (Fig. 3*b*). The perfusion times required to remove the *n*-alkanols increased progressively with increasing chain length and decreasing water solubility.

It has been shown that short-chain alkanols (C_2–C_8) block nerve impulse conduction in the squid giant axon by inhibiting the increase in sodium conductance that is associated with the nerve impulse[14]. This also seems the most likely explanation for the action of the alkanols on the LOT, especially as their effects were similar to the action of tetrodotoxin, a specific sodium-channel blocker. However, nerve block would also be predicted if the alcohols acted by an inhibition of the sodium pump[15]. To test this, nerves were exposed to the sodium-pump inhibitor ouabain (10–15 μM)[16]. Low concentrations of ouabain (10 μM) produced a reversible block of the action potential within 20 min. The rate of decline of the action potentials could be accelerated by repetitive stimulation, as one would expect if this effect were due to block of the sodium pump. In contrast, the effects of the alkanols could not be enhanced by repetitive stimulation (not shown). It therefore seems that both short- and long-chain alkanols act directly on the sodium conductance mechanism and not on the sodium pump.

We have already noted that the long-chain and short-chain *n*-alkanols have opposing effects on membrane fluidity (Figs 1, 2), but both block nerve conduction. Thus, there is no direct correlation between the effects of the *n*-alkanols on membrane fluidity or on phase transitions and their ability to block the conduction of nerve impulses. It could, however, be argued that the decrease in fluidity caused by long-chain alkanols (C_{10}–C_{12}) is responsible for their nerve-blocking action[6], and if this were so the anaesthetic action of all the alkanols could still be regarded as the result of perturbations of the membrane lipids. This hypothesis predicts that alkanols which have opposing actions on membrane fluidity and phase transitions should also have antagonistic actions in their local anaesthetic properties[6].

This proposition was tested by studying the interaction between the local anaesthetic actions of butanol and dodecanol in the LOT. Conventional methods of isobolic analysis of drug interaction were not practicable because of the slow time course of the action of dodecanol. Instead, the depressant action of 30 mM butanol on the LOT compound action potential was measured at various times during continuous perfusion of the nerve with a weak suspension of dodecanol (0.2 mM) in artificial cerebrospinal fluid (CSF). If the actions of the two alkanols were

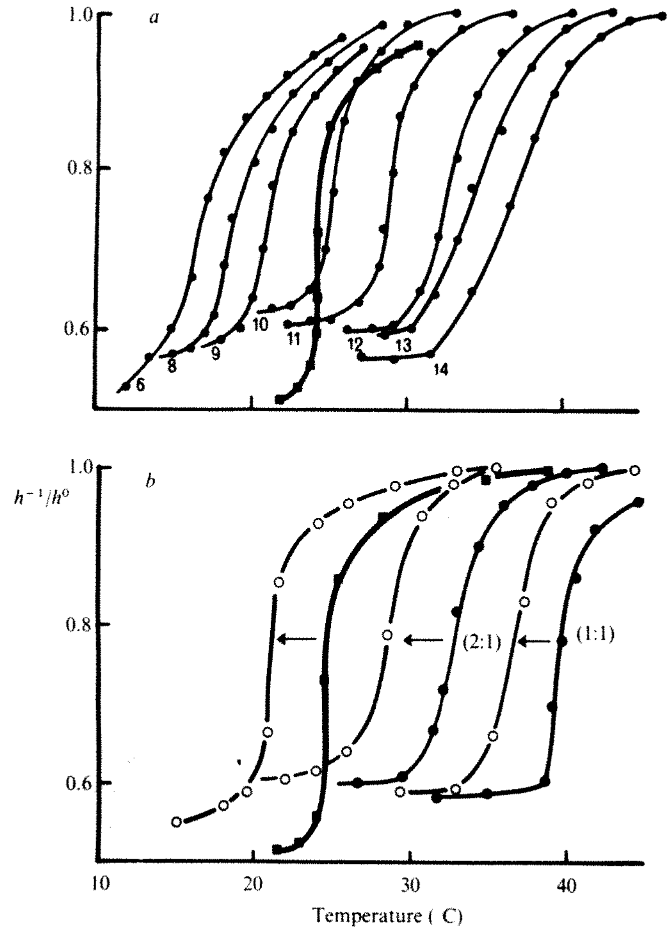

Fig. 2 *a*, The effects of *n*-alkanols on the phase transition of dimyristoyl phosphatidylcholine (DMPC). The phase transition of an aqueous suspension of pure DMPC is shown by the thick line and solid square symbols. All the other curves refer to the phase transition in the presence of alcohols; the chain length is given at the foot of each curve. The alkanols were added either as an aqueous suspension in phosphate buffer to the dried-down lipid samples with the spin label 4′,4′-dimethylaxazolidine derivative of 16-ketostearic acid as in the legend to Fig. 1, or were mixed directly with the lipid in chloroform before drying down. The final alkanol concentrations were 80 mM to C_9 or 50 mM for C_{10}–C_{14}, and the DMPC concentration was 100 mM. *b*, The effect of butanol (C_4, ○, 150 mM) and dodecanol (C_{12}, ●) on the phase transition of DMPC separately and in combination (DMPC:C_{12} at 1:1 or 2:1 molar ratios). As in *a* the DMPC phase transition curve (■) is depressed by butanol and raised by dodecanol. Butanol antagonism (arrows) of the elevation of the phase transition by dodecanol is shown for 1:1 and 1:2 ratios of dodecanol to lipid.

antagonistic, the depressant effect of the butanol would be expected to decrease as the nerve took up the dodecanol. However, the effectiveness of butanol increased progressively with the period of exposure of the nerve to dodecanol (Fig. 4) and this increase in potency was apparent long before the depressant action of dodecanol itself became evident. A similar effect, of even slower time course, was seen when a nerve was exposed to tridecanol instead of dodecanol. The increase in the potency of butanol was not attributable to deterioration of the nerve with time, because the effect of repeated applications of 30 mM butanol to nerves superfused only by artificial CSF was essentially constant over 12 h (Fig. 4).

Other chemical agents have been found to antagonise the physical effects of the short-chain alkanols on lipid bilayers and membranes. For example, the alkanols protect red cells from hypotonic haemolysis, whereas the pyridine derivative 4,*N*-ethylaminoethyl,2-methyl,3-hydroxy,5*S*-methyl-thiomethyl pyridine (EMD-21657; Merck Darmstadt) antagonises the anti-haemolytic actions of alkanols and is an effective treatment of alcoholic organic brain syndrome[41]. Although EMD-21657 alone had no effect on the transition temperature of dimyristoyl phosphatidylcholine at up to equimolar concentrations with the lipid, it slightly antagonised the effect of hexanol in lowering the transition temperature of the lipid. However, EMD-21657 consistently potentiated the local anaesthetic action of hexanol on the LOT (Fig. 5) and, by itself,

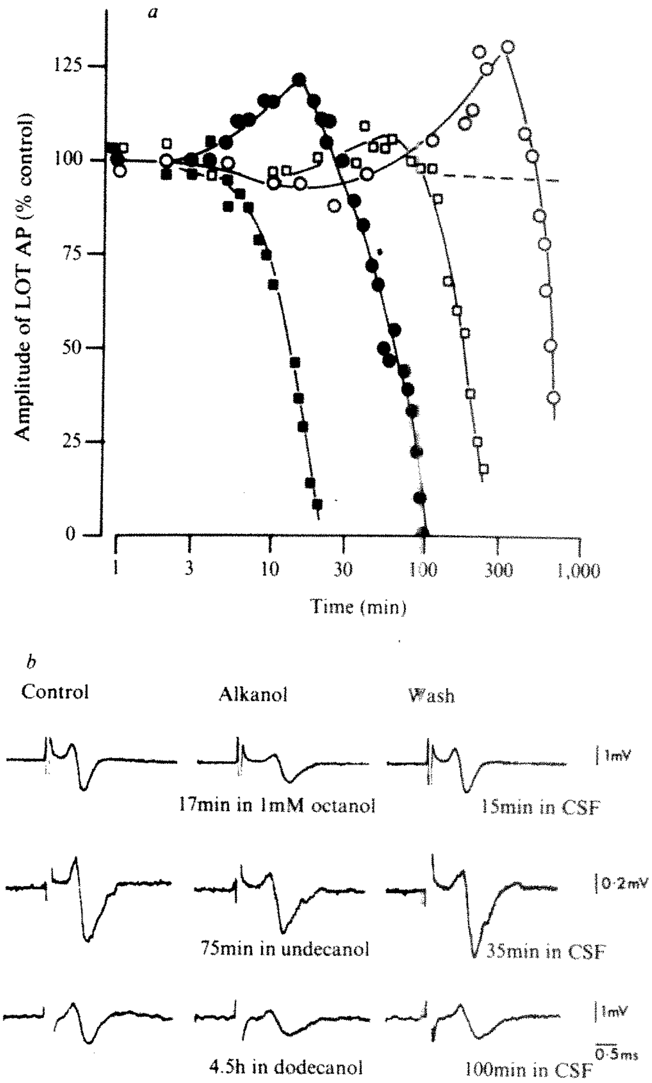

Fig. 3 Local anaesthetic action of primary alkanols. *a*, Time course of action; each alkanol was administered continuously from zero time as a solution or suspension. The ordinate represents the amplitude of the LOT compound action potential expressed as a percentage of its amplitude immediately before the administration of the alkanol. Note logarithmic time scale along abscissa. Results from four separate experiments are shown. Those of a fifth nerve exposed to CSF only are indicated by the broken line (the experimental points were omitted for clarity as were the results for butanol which were essentially similar to those for octanol). ■, C_8; ●, C_{10}; □, C_{11}; ○, C_{12}; ---, CSF. *b*, Sample records from three experiments. As the alkanols affect nerve conduction the action potential becomes smaller and broader, and occurs at a longer latency. This combination of effects is similar for all alkanols and other anaesthetics. Note that the reversal of nerve block by washing with CSF becomes slower with increasing chain length. The details of preparation and incubation have been described previously[38,39]. Octanol was administered as a 1 mM solution in artificial CSF and the long-chain alcohols were administered as suspensions of 1 mM for decanol, 0.5 mM for undecanol and 0.2 mM for dodecanol in CSF. All experiments were carried out at 38 °C with supramaximal stimuli given to the LOT at 0.2 Hz.

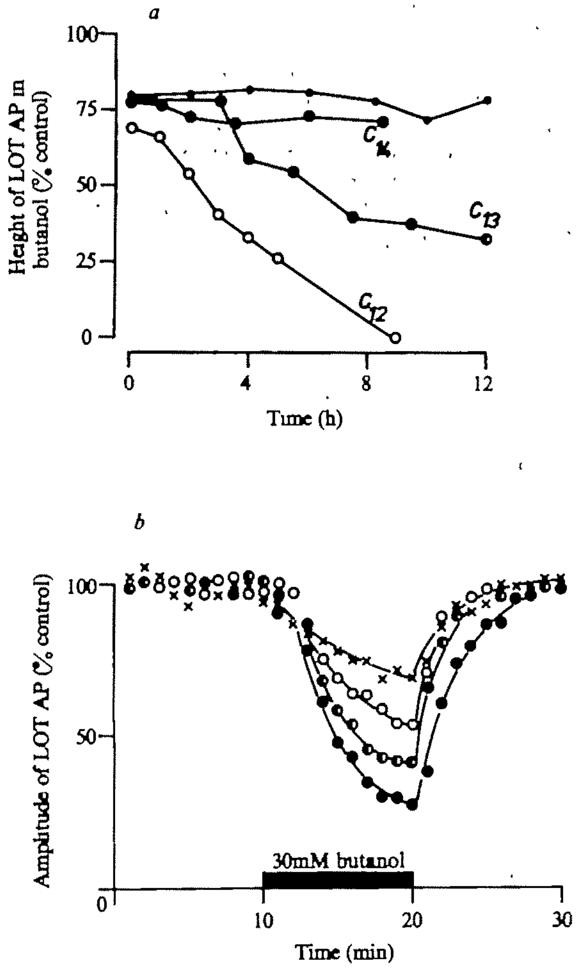

Fig. 4 Interaction between the local anaesthetic actions of butanol and long-chain alkanols (C_{12}–C_{14}). Experimental details as for Fig. 3. *a*, Four representative experiments are shown. Each nerve was exposed to 30 mM butanol for 10 min at various times while a continuous application of one long-chain alkanol was given as indicated. The ordinate represents the amplitude of the LOT action potential (AP) after 10 min exposure to 30 mM butanol expressed as a percentage of the average amplitude for the 5 min before exposure. Dodecanol (0.2 mM) and tridecanol (0.1 mM) were administered as suspensions, tetradecanol was administered at 50 μM in a liposomal suspension (molar ratio of 1:2 with egg yolk lecithin prepared as previously described[28]). ●, No addition; ⊙, C_{14}; ◐, C_{13}; ○, C_{12}. *b*, An example of the potentiating action of dodecanol on the local anaesthetic action of butanol. Initially, the nerve was exposed to 30 mM butanol to determine its sensitivity (X). After washing out the butanol the dodecanol suspension was applied continuously for the remainder of the experiment. At 2 (○), 4 (◐) and 5.5 (●) h, the sensitivity of the nerve to butanol was again determined. The progressive increase in the efficacy of butanol can be clearly seen.

had a small depressant action on the action potential at concentrations up to 5 mM, with little effect on conduction velocity. Similar results have been obtained with the frog sciatic nerve. These results indicate that the local anaesthetic actions of EMD-21657, either alone or with hexanol, cannot be correlated simply with their effects on the physical properties of lipid bilayers.

Temperature effects and unitary hypotheses of local anaesthesia

The interpretation of the effects of temperature on conduction and local anaesthetic action is complicated. The functional properties of the nerve are known to vary with temperature and therefore the sensitivity to perturbation by anaesthetics will be difficult to predict. Cooling the LOT from 38 to 28 °C in the absence of any anaesthetics slowed conduction without a significant reduction in the amplitude of the compound action potential or increase in its threshold. Further cooling slowed conduction still more, decreased the amplitude of the action potential and increased the threshold until at 13–15 °C, cold block occurred. At the same time, the partition coefficient of the anaesthetic in excitable membranes is likely to be changing. For example, the partition of butanol and benzyl alcohol into various lipid bilayers and erythrocyte membranes decreases with cooling (Fig. 6); this is a further complication in predicting how anaesthetic potency will vary with temperature. However, in spite of these complications, the changes in potency of these two local anaesthetics with temperature are inconsistent with any simple unitary hypothesis based on perturbations of the lipid bilayer; suggestions raised include:

(1) Fluidity changes. The fluidity of the lipid bilayer decreases with decreasing temperature and it might be expected that the anaesthetic potency of both alcohols would also decrease, because their fluidising effect would be antagonised by the reduction in temperature (see Fig. 1), and because their concentrations in the membrane would also be reduced. In fact, the potency of benzyl alcohol as a nerve-blocking agent increases with cooling, so that 10 mM benzyl alcohol attenuated the LOT compound action potential by 30–40% at 37 °C, by 60–75% at 27 °C, and by 80–90% at 20 °C (Fig. 6), whereas the potency of butanol decreased slightly over the same temperature range. These marked differences in the effects of butanol and benzyl alcohol with temperature cannot be rationalised simply in terms of an unitary hypothesis based on fluidity perturbations of the lipid bilayer.

(2) Phase separations. Two recent hypotheses propose that anaesthetics act by fluidising regions of phospholipid which are normally in the liquid-crystalline state. Trudell[17] proposed that when this occurs in the lipid bilayer, the lateral pressure the lipid exerts on the target membrane proteins increases and results in the inhibition of their functions and hence anaesthesia. Alternatively, Lee[18] proposed that the proteins responsible for nerve conduction are maintained in an optimal conformation by an annulus of rigid lipid molecules (similar to a liquid-crystalline state) and that anaesthetics fluidise this lipid to cause the protein to relax into an inactive conformation. Both hypotheses predict that cooling a nerve blocked by an anaesthetic should restore conduction. Furthermore, as nerve block is produced by concentrations of alkanols that decrease the T_t of phospholipid bilayers by 3–5 °C, Lee's hypothesis predicts that cooling a nerve by that amount should restore nerve conduction. Both 50 mM butanol and 15 mM benzyl alcohol block conduction in the LOT and produce a decrease in the T_t of phospholipid bilayers of about 3 °C. However, in neither case does cooling by this amount restore nerve conduction (see Fig. 6 and above). Thus,

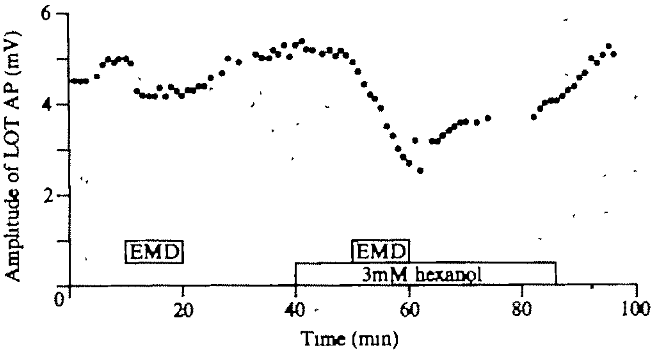

Fig. 5 Interaction between EMD-21657 and hexanol on the amplitude of the LOT compound action potential. EMD-21657 (5 mM) has a slight depressant action but sharply potentiates the action of 3 mM hexanol (3 mM hexanol alone depressed the LOT action potential by 15% in this preparation after 20 min application).

the simplest forms of these two unitary hypotheses are not supported experimentally, and in our view, the wide range of temperatures over which conduction occurs in the LOT and other nerves is a clear indication that perturbation of a critically balanced phase separation is not a likely mechanism of local anaesthetic action. In particular, it is significant that the LOT will conduct at temperatures well above the physiological temperature of 38 °C, at least up to 42 °C, when the annular lipids would be considerably fluidised.

(3) Bilayer thickness. The present data are clearly not directly concerned with the question of changes in bilayer thickness as a mechanism of anaesthetic action, as proposed by Ashcroft *et al.*[19,20] and Haydon *et al.*[15,20]. However, both butanol and benzyl alcohol would be expected to decrease bilayer thickness as they expand the area of red cell membranes[22] and decrease the gel–liquid phase transition temperatures of artificial phospholipid bilayers[23]. This view is consistent with the evidence that anaesthetics decrease the thickness of phospholipid monolayers as their area expands[24,25], but contrasts with the evidence from capacitance measurements on black lipid membranes[19–21]. Even so, we would expect benzyl alcohol and butanol to have similar effects on bilayer thickness and that their potency as local anaesthetics would vary with temperature in the same direction if their primary action were to be on membrane thickness. The fact that this is not observed experimentally suggests that any changes in bilayer thickness that are produced by alcohols will not correlate directly with their local anaesthetic action.

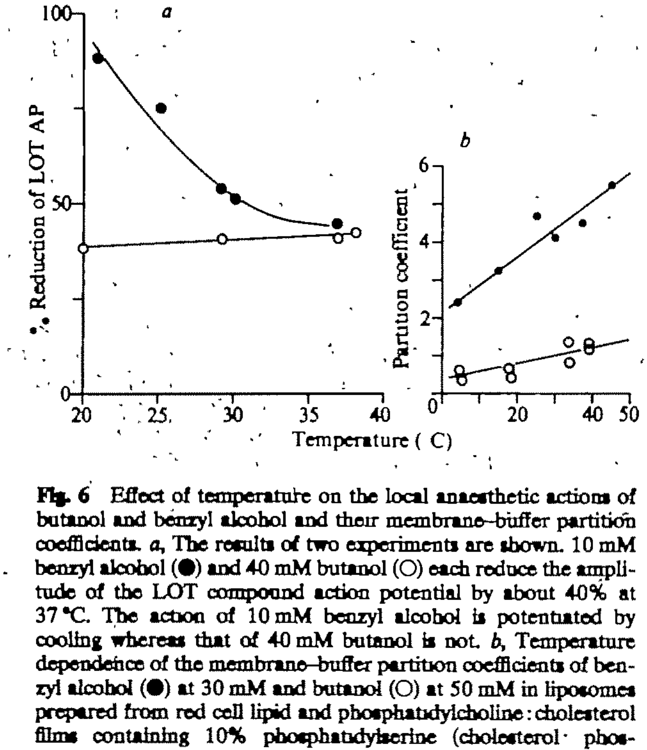

Fig. 6 Effect of temperature on the local anaesthetic actions of butanol and benzyl alcohol and their membrane–buffer partition coefficients. *a*, The results of two experiments are shown. 10 mM benzyl alcohol (●) and 40 mM butanol (○) each reduce the amplitude of the LOT compound action potential by about 40% at 37 °C. The action of 10 mM benzyl alcohol is potentiated by cooling whereas that of 40 mM butanol is not. *b*, Temperature dependence of the membrane–buffer partition coefficients of benzyl alcohol (●) at 30 mM and butanol (○) at 50 mM in liposomes prepared from red cell lipid and phosphatidylcholine : cholesterol films containing 10% phosphatidylserine (cholesterol : phospholipid ratio 1 : 2), respectively. (Data for benzyl alcohol from ref. 40.) The partition coefficients for butanol were determined using equilibrium dialysis with estimation of butanol by gas chromatography.

A degenerate protein perturbation hypothesis

The results presented here show that it is very unlikely that there is a correlation between anaesthetic effects and any of the physical properties of lipid bilayers that have been proposed as the basis of unitary mechanisms of local anaesthetic action. We must therefore conclude that anaesthetics do not generally act by perturbing the lipid bilayer regions of membranes and that direct interactions between the anaesthetic molecules and target membrane proteins are responsible for local anaesthesia. However, we do not imply that all anaesthetics act by binding to a specific site on one or more of the functional membrane proteins; this is extremely unlikely. Instead, we suppose that, for example, small molecules such as halothane or cyclopropane are distributed in one set of hydrophobic sites of appropriate dimensions within the protein, and that larger molecules such as barbiturates, steroids and aromatic amines, which show varying degrees of stereoselectivity[26–29], will bind to distinct sets of sites on the target membrane proteins.

There is some experimental evidence for selectivity in the binding sites of amine local anaesthetics for their action on nerve conduction. For example, their inhibition of the sodium current depends on both the rate of stimulation[30] and the size of the voltage step applied to nerves under voltage clamp[31]. This suggests that they bind to a precise part of the sodium conductance mechanism which must possess the appropriate conformation for binding to occur and which may be located within the channel itself. Finally, we suggest that any molecule with structural features similar to those of phospholipids, such as dodecanol, will compete for the hydrophobic regions of membrane proteins which normally interact with the chains of membrane phospholipids, and that replacing the annular lipids[32] with anaesthetic molecules interferes with the normal lipid–protein interactions that are required for the maintenance of function[33]. The evidence for this type of 'annular displacement mechanism' will be presented elsewhere[34]. Thus, we propose that the binding of anaesthetics to many different sets of sites on the target proteins can inhibit their function and that the mechanism of anaesthetic action is therefore highly degenerate at the molecular level. We term this the degenerate perturbation hypothesis[6,35].

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Multiplicity of germline genes specifying a group of related mouse κ chains with implications for the generation of immunoglobulin diversity

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The number of germline genes specifying the variable sequences of the $V_\kappa 21$ group of light chains was determined by saturation hybridisation analysis to be four to six. This gene multiplicity is considerably less than the total variability in the group, indicating that κ-chain diversity is generated by somatic mutations in both framework and complementarity-determining (hypervariable) regions.

THE extensive repertoire of immunoglobulins can be accounted for in two ways: multiplicity of germline variable-region genes (V_L and V_H) and somatic diversification of V genes. In the case of mouse λ light chains, the pattern of variability is simple[1]. Nucleic acid hybridisation and gene-sequencing studies indicate that variability arises somatically by mutation of one germline V_λ gene[2-4]. In contrast, for κ chains, which constitute the bulk of the light chains in the mouse, the pattern of variability is extensive, and can be explained at least in part by multiple germline genes. The V_κ regions fall into various subgroups that are defined by characteristic amino acid residues and shared sequence insertions or deletions[5]. As multiple, identical somatic mutations would be required to produce V_κ regions of two or more subgroups from a single V_κ gene, it has been inferred that different subgroups are coded for by different germline genes. The idea of multiple germline V_κ genes is consistent with the results of nucleic acid hybridisation studies, which indicate that the messenger RNAs coding for unrelated κ chains have little or no homology in their V-region sequences, and that each of these different species of κ-mRNA seems to be coded for by 2–5 genes per haploid genome[6-12].

In this study we assess the extent to which somatic mutation might also contribute to V_κ diversity. We have approached this problem by determining the germline gene multiplicity for a group of related κ chains. This group, called $V_\kappa 21$ according to the recent classification of mouse V_κ sequencies[13], is composed of 30 examples of κ chains produced by independently induced plasmacytomas of either NZB or BALB/c mice (see accompanying paper[14] and ref. 15). The amino-terminal sequences (positions 1–23) of these κ chains are identical except for a single substitution. Throughout the rest of the V region (positions 24–112) there is considerable diversity. Among the 22 NZB examples of $V_\kappa 21$, 20 unique sequences have been found with differences both in the framework and complementarity-determining (hypervariable) regions.

Our estimate of the number of genes coding for selected $V_\kappa 21$-mRNAs indicates that there are only four to six germline V genes for the entire $V_\kappa 21$ group, a value far too small to account for the diversity of this group. This provides a compelling argument for the importance of somatic processes in the generation of κ-chain diversity. Moreover, these results indicate that such somatic processes can result in extensive substitutions in the framework region as well as in the complementarity-determining regions.

Determination of the nucleotide sequence homology among κ-mRNAs

The number of $V_\kappa 21$ genes can be determined by nucleic acid hybridisation measurements with probes containing V-region sequences of selected $V_\kappa 21$-mRNAs. To interpret such measurements correctly, it is necessary to know the capacity of a particular V probe to anneal to various V-region sequences within and outside the $V_\kappa 21$ group. For this reason, we first examined the cross-hybridisation between a labelled complementary DNA representing one member (MOPC 321) with three other selected mRNAs of the group, those coding for PC 3741, PC 7043 and PC 2880 κ chains. These four light chains

Table 1 Cross-hybridisation of full-length MOPC 321 κ-cDNA with κ-mRNAs

mRNA	% protection of full-length MOPC 321 κ-cDNA	% of amino acid homology in the variable region
κ(MOPC 321)	97	100
κ(PC 3741)	98	>94
κ(PC 7043)	98	85
κ(PC 2880)	92	80
κ(MPC 11)	64	~50
C_κ(MPC 11)	68	0
λ(MOPC H2020)	3	
α(MOPC H2020)	3	
None	2	

Hybridisation reactions were carried out at 70 °C in 50-μl reaction mixtures[18] containing 10 ng of mRNA and 0.4–0.5 ng of ^{3}H-cDNA. All mRNAs except C_κ were purified by two cycles of sucrose gradient sedimentation. The C_κ-mRNA was purified by sucrose gradient sedimentation and electrophoresis on a formamide–polyacrylamide gel. The reactions were carried out to a C_rt of 5×10^{-2} mol l^{-1} s and assayed with S_1 nuclease as described previously[18]. The percentages of protection are corrected for 2% self-annealing of the probe at the longest incubation period. The percentages of amino-acid homology in the variable regions refer to the first 98 N-terminal amino acids[14].

differ from each other in the framework as well as the complementarity-determining regions[14]. Relative to MOPC 321 κ chain, the amino-acid sequence homologies in the variable regions of the PC 3741, PC 7043 and PC 2880 κ chains are >94%, 85% and 80%, respectively (Table 1). The variable sequences of MOPC 321 and PC 2880 are among the most distantly related in the $V_κ21$ group. For an example of an unrelated $V_κ$ sequence we used the mRNA coding for MPC 11 κ chain, a member of the $V_κ19$ group, which differs from the MOPC 321 κ chain at 10 of the first 23 N-terminal residues and about half of the total variable-region positions[5,16]. As a control for null hybridisation of V-region sequences we used an mRNA derived from MPC 11 cells ($C_κ$-mRNA), which lacks the sequences coding for the variable region of the κ chain[17].

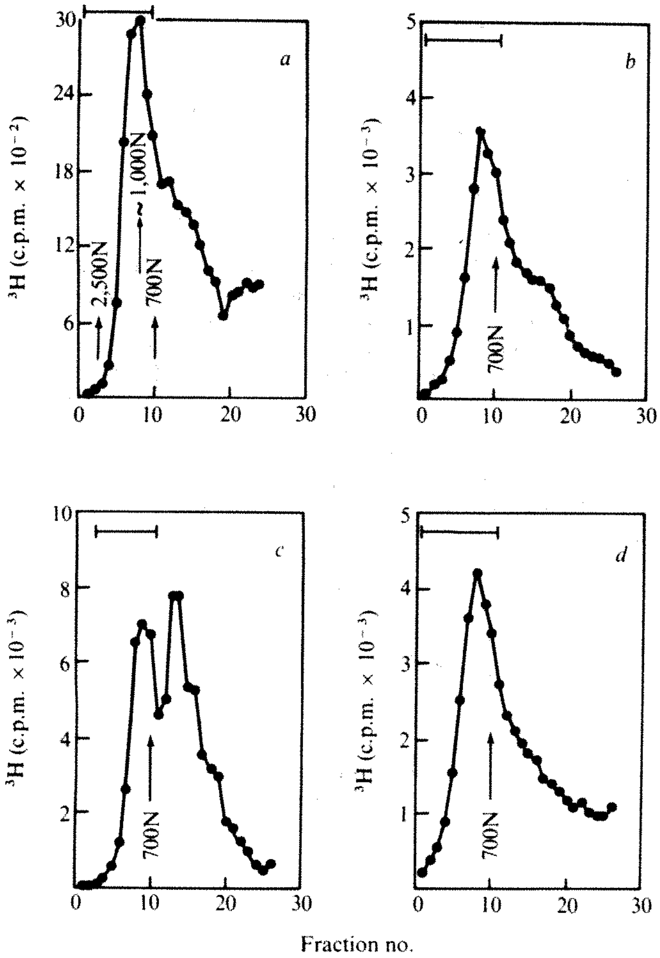

Fig. 1 Isolation of full-length κ-cDNA in alkaline sucrose gradients. 300–500 ng of ^{3}H-cDNAs were sedimented through 5–20% alkaline sucrose gradients in a SW50.1 rotor, at 36,000 r.p.m., at 22 °C for 16 h. Fractions of 200 μl were collected and the radioactivity determined by counting 5–10-μl aliquots in a scintillation counter. Arrows indicate the position of polyoma DNA fragment markers (2,500 and 700 nucleotides (N) long) analysed in parallel gradients. Panels *a–d* show the profiles of the total cDNA preparations synthesised from MOPC 321, PC 2880, PC 3741 and MPC 11 κ-mRNAs, respectively. The fractions corresponding to full-length cDNAs (horizontal bars) were pooled, ethanol precipitated, and used for subsequent hybridisation studies.

The mRNAs used in these experiments were extracted from membrane-bound polyribosomes and purified by oligo dT-cellulose chromatography, sedimentation through successive sucrose gradients and, in some cases, electrophoresis in formamide–polyacrylamide gels[18–20]. Their purity, evaluated by kinetic complexity analysis or oligonucleotide fingerprinting and 5′-terminal analysis, ranged from 75% to close to 100% (refs 18–20). The ^{3}H-cDNAs (10 c.p.m. per pg) were synthesised with avian myeloblastosis virus reverse transcriptase and oligo dT primer in conditions which yield a high proportion of full-length product. Analyses of the cDNAs on alkaline sucrose gradients showed distinct peaks at ~1,000 nucleotides and a trailing of shorter, incomplete transcripts (Fig. 1). To maximise the equivalent representation of 3′ (C region) and 5′ (V region) sequences we used only the cDNA in the fastest sedimenting (1,000-nucleotide peak) fractions. The probes were further purified by back hybridisation to their mRNA templates in conditions in which only the most abundant sequences would anneal[18]. When rehybridised to the mRNA templates at C_rt values of about 10^{-2} or greater, 90–100% of the purified cDNA formed S_1 nuclease-resistant duplex.

When full-length MOPC 321 κ-cDNA was hybridised to κ-mRNAs of MOPC 321, PC 3741, PC 7043 and PC 2880, 97%, 98%, 98% and 92% of the probe, respectively, was incorporated into S_1 nuclease-resistant hybrid (Table 1). The high proportions of MOPC 321 κ-cDNA protected from S_1 nuclease digestion by the heterologous κ-mRNAs indicate that the four mRNAs have a high degree of nucleotide sequence homology. In contrast, the MOPC 321 κ-cDNA probe was not protected by MOPC H2020 λ- or MOPC H2020 α-mRNAs, thus verifying the high specificity of the cDNA preparation. When the MOPC 321 κ-cDNA was hybridised to MPC 11 κ-mRNA or to $C_κ$-mRNA, approximately 65% of the probe was protected. A similar result was obtained when a full-length MPC 11 κ-cDNA probe was annealed to MOPC 321 κ-mRNA (data not shown). This extent of hybridisation is attributable to a common 3′ portion of the mRNA molecules, which contains the coding sequences for the constant regions of the immunoglobulin chains. Assuming that the 3′ untranslated sequences of the κ-mRNAs are identical or very similar for different κ-mRNAs, it seems reasonable to conclude that the additional 30–35% protection exhibited when the other members of the $V_κ21$ group of mRNAs were hybridised to the MOPC 321 κ-cDNA probe represents hybridisation to common variable nucleotide sequences located on the 5′ portions of the mRNA molecules. All the hybrids formed in this analysis had melting temperatures above 90 °C (ref. 19), as one would expect for relatively well matched DNA–RNA duplexes.

The above results indicate that the mRNAs specifying the various members of the $V_κ21$ group have sufficiently similar V-region sequences for their cDNA probes to hybridise effectively to all the $V_κ21$ genes in the reaction conditions used, particularly if complications of sister-strand competition are avoided (see below). On the other hand, a probe representing an unrelated V sequence, such as that corresponding to the variable portion of MPC 11 κ-mRNA, would not be expected to hybridise to a gene of the $V_κ21$ group, and conversely, a $V_κ21$ probe would not be expected to recognise the gene coding for the MPC 11 κ-variable sequence. Previous measurements of cross hybridisation between other $V_κ$ sequences have led to similar conclusions[2,21].

The high degree of homology among $V_κ21$-mRNAs suggests that nucleotide substitutions closely parallel amino-acid substitutions within the $V_κ21$ group. The κ chains of MOPC 321 and PC 2880, the two most distant $V_κ21$ members among those we have investigated, have different amino acids at 20 positions within the first 107 residues from the N-terminal end, which could be accounted for by a minimum of 23 nucleotide substitutions[14]. Assuming no other substitutions in the variable region and identity in the constant and untranslated regions of the mRNA, the minimum nucleotide divergence would be approximately 23/1,000 or 2.3% of the total mRNA sequence. Such a divergence might be manifested by a slight (2–4 °C) difference between the melting temperatures of homologous and heterologous hybrids[12], but it would not be readily detectable by the hybridisation assays used in these experiments. The absence

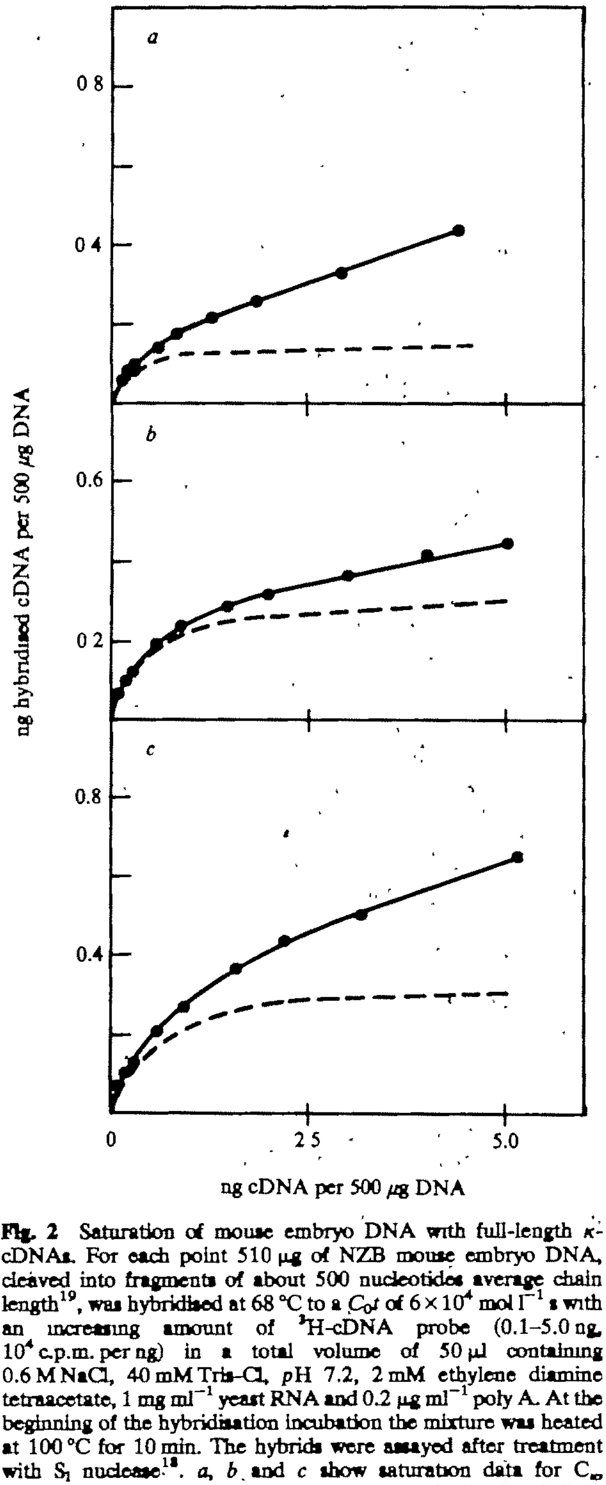

Fig. 2 Saturation of mouse embryo DNA with full-length κ-cDNAs. For each point 510 μg of NZB mouse embryo DNA, cleaved into fragments of about 500 nucleotides average chain length[19], was hybridised at 68 °C to a C_0t of 6×10^4 mol l^{-1} s with an increasing amount of ^{3}H-cDNA probe (0.1–5.0 ng, 10^4 c.p.m. per ng) in a total volume of 50 μl containing 0.6 M NaCl, 40 mM Tris-Cl, pH 7.2, 2 mM ethylene diamine tetraacetate, 1 mg ml^{-1} yeast RNA and 0.2 μg ml^{-1} poly A. At the beginning of the hybridisation incubation the mixture was heated at 100 °C for 10 min. The hybrids were assayed after treatment with S_1 nuclease[18]. *a*, *b* and *c* show saturation data for C_κ, MOPC 321 and PC 2880 κ-cDNAs, respectively. Solid lines represent the hybridised cDNA (corrected for 2% self-annealing of the probes) and broken lines represent hybridised cDNA after correction for contamination (see text). The C_κ-cDNA was prepared by incubating a full-length MOPC 3741 κ-cDNA with the unrelated MPC 11 κ-mRNA and purifying the hybrid by S_1 nuclease digestion. This cDNA consists of constant-region sequences plus untranslated sequences that are common to the PC 3741 and MPC 11 κ-mRNAs.

of cross-hybridisation between the variable sequences of MOPC 321 and MPC 11 κ-mRNAs suggests that between the two sets of corresponding genes there are, in addition to the divergence leading to amino-acid substitutions, substantial differences due to neutral mutations[22-25]. Such a situation is consistent with an evolutionary model in which there is early divergence of the ancestral genes for MOPC 321 and MPC 11 V_κ sequences and a more recent expansion of these individual gene sets.

Estimation of gene number

The gene multiplicity was determined by saturation experiments, in which a fixed amount of mouse embryo DNA was hybridised with increasing amounts of full-length κ-cDNA probes. When the probe is in large excess relative to the corresponding gene sequence, the elimination of reactive loci due to the annealing of (−) DNA sister strands is negligible, and one may expect that all the relevant genes will be duplexed with probe. This feature is particularly advantageous for measuring the aggregate number of closely related genes because it minimises the possibility of related but non-identical sequences being out-competed by perfectly matched (−) DNA strands. As this effect would be magnified when gene sequences are in excess, the validity of immunoglobulin gene copy numbers determined by reassociation kinetic analysis has been questioned[2,26,27].

When 510 μg of embryo DNA was incubated to a C_0t of 6×10^4 mol l^{-1} s with increasing amounts of probe, data such as those shown in Fig. 2 were obtained. The saturation curves were apparently biphasic and did not exhibit true saturation plateaux. The biphasic nature was more clearly revealed when double-reciprocal plots of the saturation data were made (Fig. 3). All the curves consisted of a linear component representing the saturation behaviour of the predominant cDNA species, and a deviation from linearity at high cDNA inputs, which is most readily interpreted as representing the hybridisation of a small, but diverse fraction of contaminating non-κ-chain sequences, each of which is present in subsaturating amounts. This interpretation was substantiated by several facts. First, when the input of MOPC 321 κ-cDNA was 12.5 ng (2.5 times the highest input used in Fig. 2), the saturation curve still failed to approach a plateau, and, in fact, continued to rise with constant slope. Second, the levels of putative contaminating non-κ-chain sequences in the MOPC 321 and MPC 11 cDNA probes calculated from the reciprocal plot analysis (3% and 9%, respectively, see below) are roughly proportional to the levels of impurities in the respective mRNA preparations, as judged from kinetic complexity analyses[18]. Third, when saturation experiments were carried out with homogeneous κ-mRNA sequences prepared from cloned DNA, true saturation plateaux and completely linear reciprocal plots were observed (Fig. 4). Moreover, the gene numbers estimated from these saturation values did not differ significantly from the corresponding numbers obtained with enzymatically synthesised probes when corrections for the contaminating components had been made (see below).

To calculate the saturation values corresponding to each κ-chain species, we empirically assigned a percentage of contamination to each probe, which if corrected for, would result in a completely linear curve in the reciprocal plot (Fig. 3). The levels of contamination estimated by trial and error were 2.9%, 6.5%, 6.5% and 9.0% for MOPC 321 κ-cDNA, PC 2880 κ-cDNA, C_κ-cDNA and MPC 11 κ-cDNA, respectively. When these proportions of nonsaturating components were subtracted from the hybridisation values, reasonably good plateaux were obtained (broken curves, Fig. 2). The saturation values (H_∞) were then calculated from the corrected ordinate intercepts of the double-reciprocal plots (Fig. 3). The high precision of the saturation data enabled us to calculate the H_∞ values with a maximum probable error of ±10%. These values, together with the molecular weight of the mouse haploid genome and the molecular weights of the C_κ and V_κ segments, were used to calculate the number of gene copies (Table 2). One set of gene numbers was calculated using 650 nucleotides and 350 nucleotides, respectively, for the C_κ and V_κ molecular weights, based on the fact that about 65% of full-length MOPC 321 κ-cDNA (1,000 nucleotides) is protected by MPC 11 κ-mRNA or C_κ-

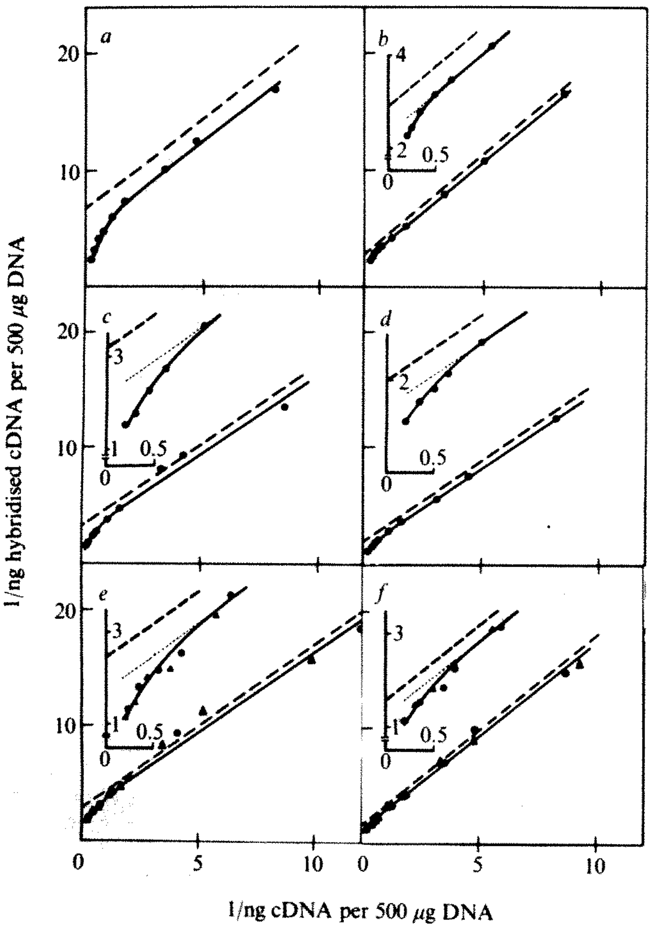

Fig. 3 Double-reciprocal plots of the cDNA saturation data. Solid lines represent hybridised cDNA (2% self-annealing subtracted). Broken lines represent hybridised cDNA after correction for contamination of probe by non-κ-chain sequences. The intercept and slope of the broken lines were estimated by the least squares method. *a–d* correspond to C_κ, κ(MOPC 321), κ(PC 2880) and κ(MPC 11) cDNA saturation reactions, respectively. *e* and *f* correspond to equimolar mixtures of κ(MOPC 321)+κ(PC 2880) and κ(MOPC 321)+κ(MPC 11) cDNAs, respectively. ▲ and ● refer to two independent experiments. Contaminant correction for the mixtures was made using an average for the individual probes. The portions of the curves with the higher cDNA inputs are enlarged in the insets.

mRNA sequences (Table 1). An alternative set of gene numbers was calculated using 521 and 479 nucleotides, respectively, for the C_κ and V_κ segments, based on current ideas of the structure of a κ-mRNA[28]. With this information, the calculated gene multiplicities per haploid genome are as follows: 2–3 genes for C_κ; 4–6 V_κ genes for MOPC 321; 4–5 V_κ genes for PC 2880; 8–11 V_κ genes for MPC-11. It is evident that all the tested V_κ genes are in a higher number of copies than the C_κ gene.

When a 1:1 mixture of MOPC 321 and MPC 11 κ-cDNAs was used, the measured number of V_κ genes is in good agreement with the sum of the individual gene reiteration values. On the other hand, when a mixture of MOPC 321+PC 2880 κ-cDNAs was used, no additive hybridisation was observed; the measured number of V_κ genes for this mixture is not significantly different from either of the individual V_κ-gene reiteration values for MOPC 321 or PC 2880. These results support the inferences made on the basis of the cross-hybridisation experiments. The additivity exhibited by MOPC 321 and MPC 11 V_κ sequences demonstrates that the two probes are hybridising to separate genetic loci, thus implying two different sets of genes. The non-additivity displayed by MOPC 321 and PC 2880 V_κ sequences indicates that both probes are hybridising to the same 4–6 V_κ21-related loci.

The saturation data with the cloned DNA sequences were also used to calculate gene numbers. In this case, estimates of the sizes of the C_κ and V_κ segments were made from measurements of the amounts of cloned MPC 11 κ-DNA sequence protected by MOPC 321 κ-mRNA (70%) and MPC 11 κ-mRNA (100%). As the cloned MPC 11-κ-sequence was calculated to be 700 nucleotides (U. Schibler, personal communication), 70% or 490 nucleotides of the probe represent C_κ sequences and 30% or 210 nucleotides represent V_κ sequences. With these parameters, gene copy numbers of 2 and 8 per haploid genome for C_κ and MPC 11-related V_κ genes were calculated (Table 2). These values are in excellent agreement with the values obtained with enzymatically synthesised cDNAs after corrections for the non-κ-chain contaminants had been made, thus indicating that such corrections do not contribute significant errors to our estimates of gene number.

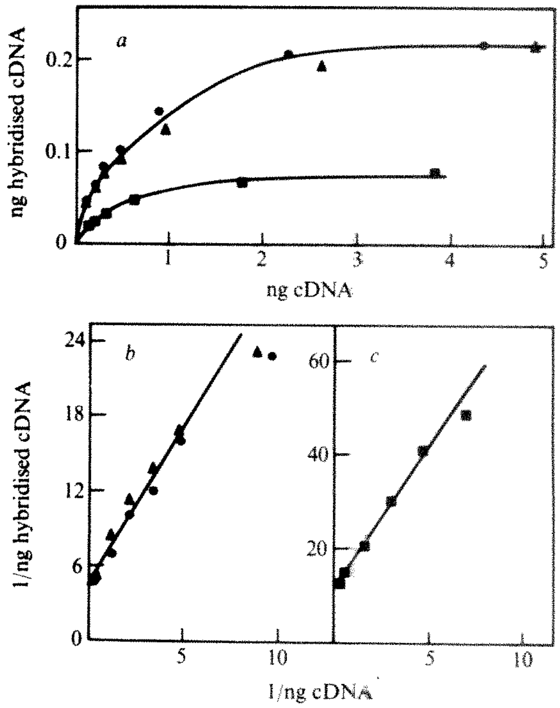

Fig. 4 Saturation–hybridisation reactions with cloned MPC 11 ^{125}I-κ-cDNAs. The cloned MPC 11 κ-mRNA sequence is carried as a 700-nucleotide insert in the plasmid pMB9. The recombinant DNA was iodinated to a specific activity of 60 c.p.m. per pg, denatured and hybridised in 80% formamide for 1 h at 50°C to an excess of MPC 11 κ-mRNA. The hybridisation mixture was treated with S_1 nuclease, and the ^{125}I-κ-DNA sequences recovered and submitted to a second round of purification by hybridisation with either MPC 11 κ-mRNA or MOPC 321 κ-mRNA and subsequent treatment with S_1 nuclease[19]. The resultant homogeneous probes represent C-plus-V-region sequences or predominantly C-region sequences, respectively. 0.448 mg of mouse embryo DNA was hybridised to increasing amounts of cloned probes (0.1–5.0 μg) in a total volume of 50 μl. The reactions were incubated and assayed as described in Fig. 2. The final C_0t value in this set of reactions was 5.4×10^4 mol l^{-1} s. *a*, Saturation curves for MPC 11 κ-DNA (▲ and ● refer to two independent experiments) and for C_κ-DNA (■). The hybridisation values were corrected for self-annealing (6% for MPC 11 κ-DNA and 15% for C_κ-DNA). *b*, *c*, Double-reciprocal plots of the MPC 11 κ-DNA and C_κ-DNA saturation curves, respectively.

The basis of $V_\kappa 21$ diversity

The $V_\kappa 21$ group of immunoglobulins is apparently represented in germline DNA by four to six genes specifying the variable-region sequences. These same four to six genes are recognised by sequence probes corresponding to any member of the group, even variants such as MOPC 321 and PC 2880 which must differ, as inferred from their amino acid sequences, by at least 7% of the nucleotides in the V-region sequence. In contrast, amino-acid sequence studies[14,15] have revealed extensive diversity in the $V_\kappa 21$ chains; if only the NZB $V_\kappa 21$ sequences are compared (to preclude any contribution of genetic polymorphism), 20 different sequences are found in the region 1–112. From the pattern of variability, $V_\kappa 21$ may be subdivided into six subgroups, $V_\kappa 21$ A to F, on the basis of two or more $V_\kappa 21$ regions sharing at least three residues that distinguish members of a particular set of $V_\kappa 21$ sequences from any other $V_\kappa 21$ sequence. The sequences that define $V_\kappa 21$A–F extend only to residue 98; the rest of what has conventionally been called V_L gene (to residue 112) shows a distinctive pattern of variability that is independent of the pattern over 1–98. It has been proposed that $V_\kappa 21$ is coded for by at least six germline genes coding for residues 1–98 or 99. The rest of the V region (99 or 100 to 112) is coded for by a separate set of genes, termed J genes. This view of $V_\kappa 21$ gene organisation is analogous to that recently proposed for the mouse λ gene[4,29].

Table 2 Gene copy numbers estimated from saturation–hybridisation experiments

cDNA	H_∞	$n(a)$	$n(b)$	$n(c)$
Constant genes				
C_k	0.15	2.4	3.0	
cloned C_k	0.08			2.0
Variable genes				
κ(MOPC 321)	0.35	6.1	4.4	
κ(PC 2880)	0.31	4.9	3.6	
κ(MPC 11)	0.49	10.6	7.7	
cloned κ(MPC 11)	0.22			8.1
κ(MOPC 321)+κ(PC 2880)	0.34	5.9	4.3	
κ(MOPC 321)+κ(MPC 11)	0 63	14.9	10.8	

H_∞ is the mass of cDNA (in ng) hybridised at infinite input, obtained from the ordinate intercepts of Fig. 3. The number of genes, n, is calculated assuming that the probes contain C_κ and V_κ sequences, respectively, comprising: a, 650 and 350 nucleotides; b, 520 and 480 nucleotides; c, 490 and 210 nucleotides. For constant genes, $n = (H_\infty^C \times MW_g)/(A \times MW_C)$. For variable genes, $n = (H_\infty^{V+C} - H_\infty^C)MW_g/(A \times MW_V))$. H_∞^C and H_∞^{V+C} are the H values for the C and C+V probes, respectively. MW_g, MW_C and MW_V are the molecular weights of the mouse genome ($1\ 8 \times 10^{12}$), the C_κ and the V_κ sequences, respectively. A is the mass of mouse embryo DNA per reaction (0.45 or 0.51 mg).

The estimate of six germline $V_\kappa 21$ genes based on shared amino-acid sequences is consistent with our finding that $V_\kappa 21$ is represented by at most six germline genes. The additional diversity in $V_\kappa 21$, over and above that due to multiple $V_\kappa 21$ genes, must be due to somatic diversification. It is plausible that one source of such diversification is joining of different $V_\kappa 21$ genes with different J genes. So far, amino-acid sequencing data suggest that such a process involves only two gene segments. However, $V_\kappa 21$ examples might still be found with other identical amino-acid substitutions which would define new subgroups. In this case, additional J-type segments would need to be invoked[30].

Extensive diversity is also observed in comparisons of $V_\kappa 21$ sequences in the region 1–98. Most of the sequence differences occur in the complementarity-determining regions. In this sense the pattern of variability is similar to that found in mouse V_λ, in which variability is confined to the complementarity-determining regions[1]. That the observed variability is concentrated in these regions could be due to somatic mutation and selection by antigen of mutants with appropriate specificities. In contrast to V_λ, however, variability in the 1–98 region of $V_\kappa 21$ is also seen outside the complementarity-determining regions, that is, in the framework regions. The 10 different framework sequences seen in the limited survey already exceeds the six that we can account for by different germline genes. That somatic variation is observed in the framework portions could imply that variation in the framework can also serve to modify antibody specificity. Alternatively, somatically derived variation both in the complementarity-determining and framework regions might reflect features of the rate or specificity of the somatic process.

The degree to which somatic diversification contributes to total V_κ-chain diversity can be assessed from our method for counting germline genes. We have shown that four to six germline genes code for $V_\kappa 21$ and about eight code for an unrelated group represented by the MPC 11 κ chain. Similar values have been obtained for the MOPC 21 κ chain, a member of the $V_\kappa 15$ group, which is unrelated to either the $V_\kappa 21$ examples or MPC 11 (ref. 12). If each V_κ group is coded for by four to eight genes, then the total germline gene content for V_κ will be four to eight times the number of such unrelated V_κ groups. This latter value has been estimated to be about 50, based on the repeat frequency of identical sequences in the region 1–23 found in a group of randomly chosen NZB κ chains[1]. Hence, the total number of V_κ germline genes can be estimated to be about 300. If the germline gene content for V_H is correspondingly high, then the potential germline repertoire of antibody specificities could be of the order of 10^5. This value, although large, still falls short of the estimated repertoire ($>10^6$) that an inbred mouse seems to express[26]. Hence, somatic diversification, as shown here for $V_\kappa 21$, must also be responsible for producing most antibody specificities.

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Rearrangement of genetic information may produce immunoglobulin diversity

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The nearly complete amino-acid sequences of 22 closely related immunoglobulin κ variable (V_κ) regions from the inbred NZB mouse are presented. This group of V_κ regions is encoded by at least six germline V_κ genes. These data also suggest that the mouse κ gene is divided into three segments termed V or variable (residues 1 to 98 or 99), J or joining (residues 99 or 100 to 112) and C or constant (residues 113–219). Tonegawa et al. *have recently described a similar J segment for mouse λ chains. Inbred mice contain multiple V_κ and J_κ gene segments. Therefore, different combinations of V and J gene segments may be joined at the DNA level during the differentiation of individual lymphocytes to contribute to antibody diversity.*

RECENT structural studies on antibody molecules, mRNAs and genes have contributed substantially to our understanding of antibody diversity. Antibody polypeptides are divided into two regions, an N-terminal variable (V) region and a C-terminal constant (C) region. The organisation of antibody genes seems unusual in two respects: (1) the V and C regions are encoded by separate genes in the germline of each organism[1,2] and (2) these genes are rearranged with respect to one another during the differentiation of the antibody-producing cell[3,4]. Variable regions, which encode the antigen-binding sites, are the focus for three theories of antibody diversity. The germline theory contends that antibody variability is encoded by large numbers of germline V genes[5]. The somatic mutation theory postulates that diversity arises by somatic variation of relatively few germline genes[6]. A third model suggests that the rearrangement of antibody gene segments during differentiation may also generate antibody variability[7–9]. The studies described here on mouse κ chains stress the importance of multiple germline V genes and suggest that antibody diversity may be increased by the combinatorial joining of multiple V and J gene segments.

Diversity in mouse light chains

Myeloma tumours artificially induced in two inbred strains of mice, BALB/c and NZB, have provided homogeneous immunoglobulins, mRNAs and chromosomal DNA for the analysis of the diversity patterns of V regions in the two light-chain immunoglobulin families, λ and κ[10]. The overall pattern of V-region diversity in mouse light chains shows that most of the variability resides in three hypervariable regions. These fold to constitute the light-chain contribution to the walls of the antigen-binding crevice. The remainder of the V region provides a scaffold for the binding site and is termed the framework region[7,11–13]. The extent and nature of diversity patterns in V_λ and V_κ are quite different.

The sequences of mouse λ chains suggest that somatic mutation may have an important role in generating antibody diversity. Amino acid sequence analyses have established that 12 of 18 V_λ regions studied are identical and that the remaining six differ by one to three amino acid substitutions, all of which occur in one of three hypervariable regions[15]. It has been proposed that the 12 identical V_λ regions ($V_{\lambda 0}$) are encoded by a single germline gene and that the variants arise by somatic mutations[6]. This interpretation is supported by nucleic acid hybridisation studies which suggest that V_λ regions are encoded by one or a few germline genes[16,17]. In addition, the DNA sequence of the putative germline V_λ gene, $V_{\lambda 0}$, has been determined for a V_λ gene isolated from presumably undifferentiated mouse embryo DNA and a variant sequence has been determined on another V_λ gene isolated from myeloma DNA[18]. (A 19th λ chain, MOPC 315, seems to differ by about 10% of its V sequence from the other 18 λ chains. This V region is probably encoded by a second V_λ gene.) Thus, most mouse λ chains exhibit a simple pattern of diversity at the protein and nucleic acid levels that is consistent with one germline V gene and variants arising by somatic mutations.

Variability in mouse V_κ regions is extensive in both hypervariable and framework regions[11,19]. A relatedness tree for the N-terminal 23 residues, a portion of the framework region, of 61 BALB/c and NZB κ chains depicts 55 different sequences (Fig. 1). We will refer to a set of V_κ regions which are very similar over their N-terminal 23 residues as a group. (Potter has suggested that each set of mouse V_k regions differing by three or fewer residues at their N terminus be given number (that is, group) designations[20]. By his criterion, 64 BALB/c V_κ sequences fall into 26 groups and 31 NZB V_κ sequences fall into 14 groups.) The complete V-region sequences from distinct groups may differ by up to 50% of their sequence. The genetic interpretation of V-region groups is that each is encoded by at least one distinct germline V gene because multiple, identical somatic mutations would be required to produce the immunoglobulin V regions of two or more groups from a single V_κ gene[21,22]. This is described as the argument of parallel mutation.

Nucleic acid hybridisation data support the supposition that different groups are encoded by distinct V genes[23]. Hence, the minimum number of germline genes in a particular antibody family can be estimated by counting V-region groups. In the mouse κ family, the number of groups has been estimated to be about 50 for either BALB/c or NZB mice[15]. If the myeloma data analysed do not constitute a random selection of the normal V_κ genes, the number of groups could be larger. In striking contrast, most λ chains fall into a single group.

The variability of mouse κ chains within a single group, $V_\kappa 21$, does not resemble that of the predominant λ group (for example, with variability only in hypervariable regions). This has been shown for eight BALB/c κ chains in the V_κ 21 group that have identical N-terminal sequences except for a single substitution. Three pairs of these V_κ regions fall into closely related sets or subgroups, designated $V_\kappa 21A$, $V_\kappa 21B$ and $V_\kappa 21C$, which are defined by multiple shared or subgroup-associated amino acid residues. The remaining two V regions differ from V_κ regions of the three subgroups and from each other[24,25]. This survey suggests that the $V_\kappa 21$ group in BALB/c mice is coded for by at

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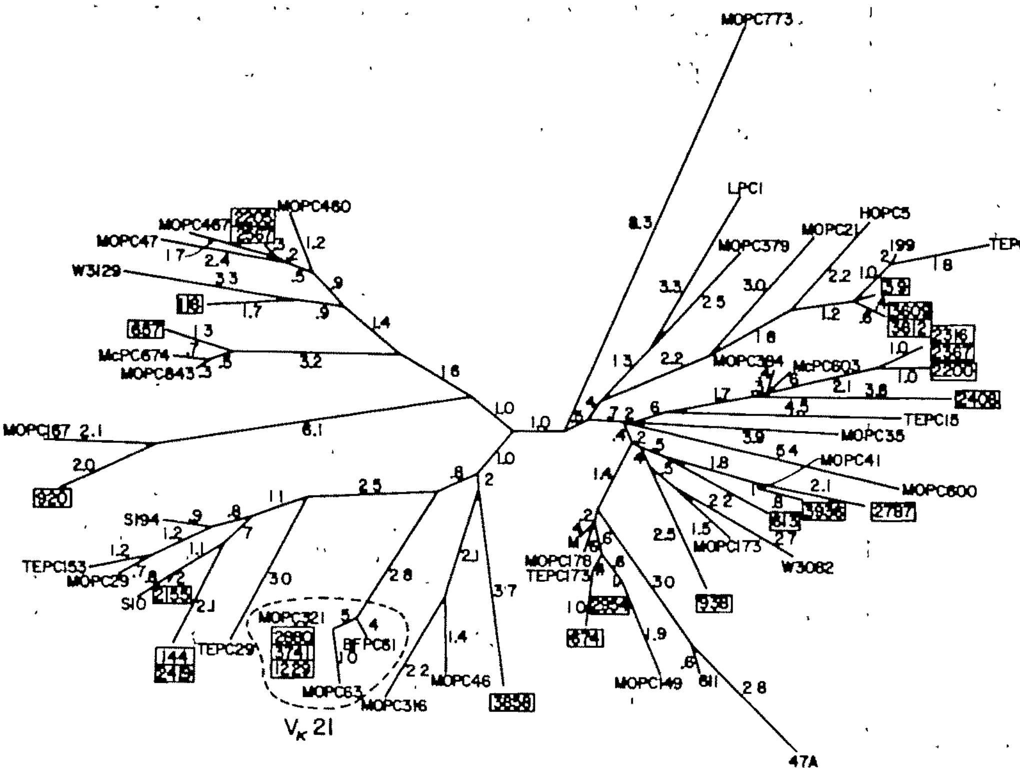


Fig. 1 A relatedness tree for N-terminal 23 residues of 55 different V_κ sequences of the mouse. These V_κ regions are derived from NZB (boxed) and BALB/c mice. The $V_\kappa21$ group is indicated by a dotted circle. The numbers on the lines indicate the number of nucleotide substitutions that separate two successive junction (node) points on the tree. Thus, the minimal extent to which individual κ chains differ from one another at the nucleotide level can be determined by summing the lengths of the lines between the two V_κ regions (adapted from ref. 19)

least three germline V genes by the argument of parallel mutation.

The object of this study is to analyse further the extent and pattern of diversity in the $V_\kappa21$ group of NZB mice. In a screen of the N-terminal sequences of 31 NZB κ chains, three $V_\kappa21$ chains, PC3741, PC2880 and PC1229, were found[26]. Using the NZB κ chains, PC3741 and PC2880, or the BALB/c $V_\kappa21$ chains, T124 and M70, antisera were made which specifically identify $V_\kappa21$ chains and distinguish a set reacting with antisera to PC2880 or M70 from a second set reacting with antisera to PC3741 or T124 (Fig. 2). Of 700 NZB myeloma immunoglobulins screened with these antisera, 5.7% fell into the first set and 3.1% into the second. In addition, the normal light chains from the sera of several inbred strains of mice crossreact with these antisera to levels of 7.2% and 1·4%, respectively.[27] Thus, the $V_\kappa21$ group constitutes about 8–9% of normal serum and myeloma light chains. This group is an ideal model system for studying the nature of diversity within the κ family of the mouse because many members of the $V_\kappa21$ group can be readily identified by rapid serological assays. Furthermore, the high frequency of these κ chains in normal sera suggests that the $V_\kappa21$ light chains are used not only in myeloma antibodies, but also in normal antibodies. In this article we report on the V-region sequences of 22 NZB κ chains belonging to the $V_\kappa21$ group. These data suggest that the $V_\kappa21$ group is encoded by multiple germline genes and that the V region is encoded by two separate DNA segments, V and J.

$V_\kappa21$ regions fall into six subgroups

Twenty-two NZB $V_\kappa21$ regions are compared. A summary of the methodology used to sequence these V regions is given in the legend to Fig. 2 and the complete data will be presented in detail elsewhere[28–30]. Of these $V_\kappa21$ regions, 18 fall into six subgroups, denoted by $V_\kappa21$A, B, C, D, E and F, in which two or more closely related V_κ regions share at least three subgroup-associated residues. Subgroup-associated residues distinguish the members of a particular subgroup from members of most of the other subgroups (boxed residues in Fig. 2); 29 subgroup-associated residues occur in positions 1–98. Variable regions from different subgroups differ from one another by 3 to 20 residues over their N-terminal 98 residues (Fig. 3). The reason for limiting our subgroup analysis to the N-terminal 98 residues will become apparent later. Again, even closely related subgroups (such as $V_\kappa21$E and $V_\kappa21$F) seem to be encoded by separate germline V genes by the argument of parallel mutation, namely, that it is unlikely that somatic mutation could generate in two or more independent lymphocyte lineages the three identical substitutions that separate V_κ regions of these two subgroups. Thus, at least one distinct germline V gene seems to encode each of the six subgroups (Fig. 3).

The minimal number of six $V_\kappa21$ germline genes deduced from V region amino acid sequences is larger than the one to three $V_\kappa21$ deduced from nucleic acid hybridisation experiments[31]. However, saturation-hybridisation studies using DNA probes derived from several $V_\kappa21$ mRNAs suggest that four to six germline genes encode the $V_\kappa21$ regions (see accompanying paper[32]). This number is consistent with the number of V genes we have identified so far by subgroup analysis.

It is possible that additional $V_\kappa21$ subgroups will be found. Four of the NZB $V_\kappa21$ regions (that is, PC2154, PC2413, PC7461 and PC2960) do not fall into any of the six subgroups and are indicated as distinct branches on the relatedness tree (Fig. 3). These V regions cannot yet be designated separate subgroups because a similar reasoning is needed to invoke the argument that separate germline genes for each subgroup avoid multiple identical somatic mutations (see above). If new $V_\kappa21$ sequences are found which pair with these examples, additional $V_\kappa21$ subgroups would be defined. In addition, currently defined subgroups may split into two or more new subgroups. For example, PC2485 was a member of the $V_\kappa21$E subgroup until the sequence of PC4039 was determined. These two identical sequences then became subgroup $V_\kappa21$F, sharing three distinct subgroup-associated residues (Fig. 2).

If additional $V_\kappa21$ subgroups are defined, then two possibilities must be considered. The saturation-hybridisation experiments have underestimated the number of $V_\kappa21$ genes. Alternatively, a somatic mechanism may exist which permits a single germline V gene to generate two or more subgroups of $V_\kappa21$ regions.

Genetic rearrangement during differentiation

The $V_\kappa21$ regions seem to have two distinct segments which we have designated the V segment (residues 1 to 98 or 99) and the J segment (residues 99–112). With this nomenclature, we can distinguish the V segment from the classical V region (residues 1–112).

The N- and C-terminal boundaries of the V and J segments are defined as follows. The J segment seems to have started by position 101 because of the invariant association of a leucine at this position with an alanine and leucine at positions 105 and 111, respectively, in the J segment of six different V$_\kappa$21 regions (Fig. 2). This repeated invariant association of three residues suggests that positions 101–111 behave as a discrete genetic unit. The last subgroup-associated residue of the V segment is at position 98. Accordingly, the N-terminal boundary of the J segment may start somewhere between residues 99 and 101. Position 100 is an amino acid insertion in one κ chain (PC7132) and we have arbitrarily chosen the J segment to include this position, suggesting that the extra residue comes from a J segment one residue longer than the others. The C-terminal boundary of the J segment occurs at position 112, the C-terminal-most variable residue in κ chains (Fig. 2. PC2413). Moreover, as the C region seems to be encoded by one or a few C genes[17,32–34], the variability in the J region is too great to be accounted for by multiple C$_\kappa$ genes beginning at position 100.

The V and J segments associate independently with one another (Fig. 4). In the 18 V$_\kappa$21 regions with relatively complete sequence data between residues 100 and 112, there are 16 different V segments and eight different J segments (Fig. 2). A particular J segment may be associated with V$_\kappa$ segments from four different subgroups (sequence 5 in Fig. 4). Conversely, identical V segments may be associated with different J segments (for example, PC6684 and PC7175, PC7043 and PC7183, and PC4050 and PC9245). The implication is that the V and J segments associate freely with one another and that particular V segments are not generally associated with certain J segments (Fig. 4). Indeed, some of these same J segments are associated with non-V$_\kappa$21 V segments of widely differing sequence[11]. Thus, a particular J segment of the κ family may be capable of associating with any V segment of the κ family.

Nucleic acid studies on mouse λ and κ genes also suggest that there are V and J segments. Two mouse V$_\lambda$ genes isolated from embryonic DNA terminate their V genes at a position homologous to residue 101 (refs 2, 18). In addition, a J$_\lambda$ segment consisting of approximately 36 nucleotides and separated from the V$_\lambda$ segment by intervening DNA sequences has been described[4]. Thus, the mouse λ-gene family also seems to be composed of V, J and C segments. Several mouse V$_\kappa$ genes isolated from myeloma DNA also terminate at approximately position 101 (ref. 35). These DNA studies taken together with our V$_\kappa$21 sequence data suggest that the κ-gene gamily has two important organisational features (Fig. 5): (1) The κ chain is encoded by three noncontinuous gene segments, V, J and C; (2) multiple V-gene and J-gene segments are present in the κ-gene family of each mouse.

The most attractive function for the J segment is that it may be involved in generating antibody diversity. Residues 100 and 101 constitute a portion of the third hypervariable region[14]. Accordingly, if the J segment occurs at positions 100–112, a

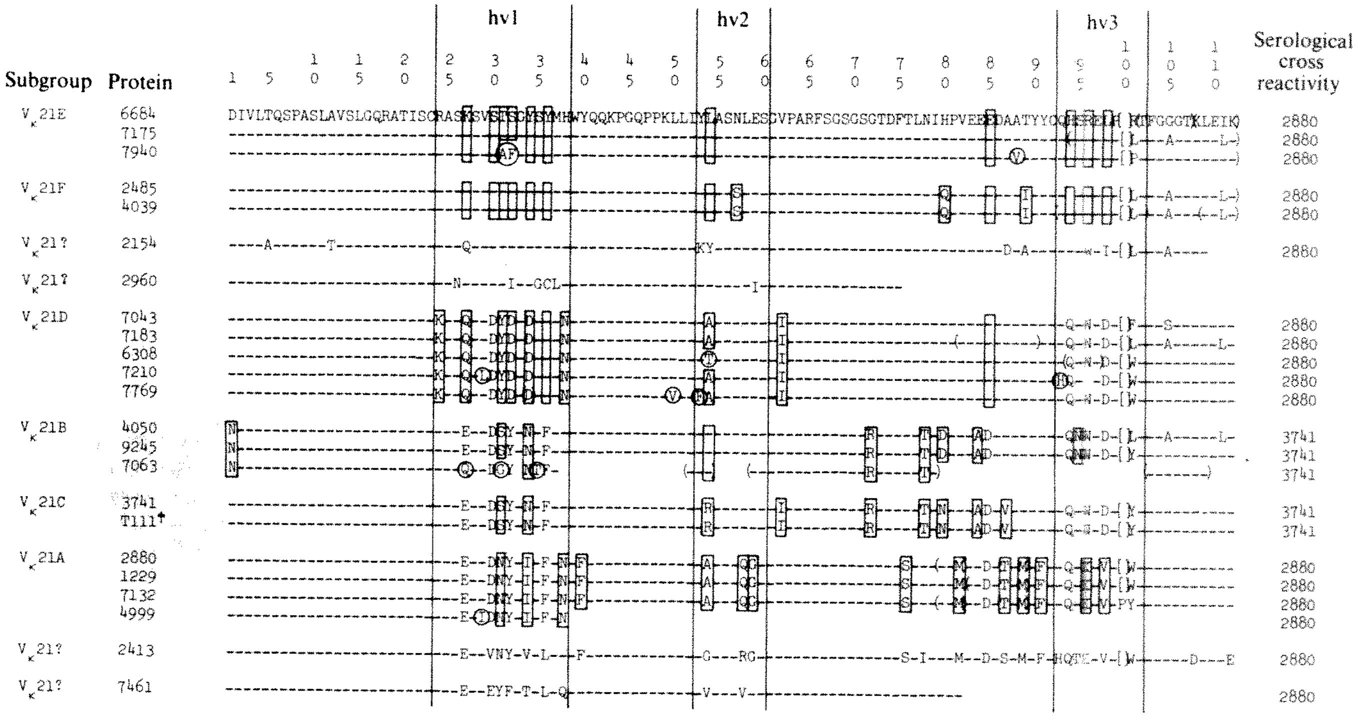

hv1
hv2
hv3
Subgroup
Protein
Serological cross reactivity
V$_\kappa$21E 6684 7175 7940
V$_\kappa$21F 2485 4039
V$_\kappa$21? 2154
V$_\kappa$21? 2960
V$_\kappa$21D 7043 7183 6308 7210 7769
V$_\kappa$21B 4050 9245 7063
V$_\kappa$21C 3741 T111†
V$_\kappa$21A 2880 1229 7132 4999
V$_\kappa$21? 2413
V$_\kappa$21? 7461
2880
3741

Fig. 2 The amino-acid sequences of V regions from the mouse V$_\kappa$21 group. The sequences are divided into subgroups which are designated V$_\kappa$21A–V$_\kappa$21F. Additional potential subgroups are indicated with question marks. The T111 sequence is a BALB/c protein[25], identical to PC3741, which permits the definition of subgroup V$_\kappa$21C. The subgroup-associated residues unique to three or fewer subgroups are boxed. The intra-subset substitutions are circled. Amino-acid compositional data are indicated by brackets. The three hypervariable regions are indicated as hv1, hv2 and hv3. The J segment extends along residues 100–112. The serological crossreactivity of individual proteins to antisera directed against two screening κ chains is given on the right. The following general strategy was used for sequencing these V regions. The intact light chain was analysed on the automatic sequenator for 40 residues. The procedures for automatic sequence analysis are given in ref. 28 where the data for a 78-residue run on PC4050 are presented. Methionine fragments were prepared; all V$_\kappa$ regions but one have a methionine at position 37. Methionine fragments starting at position 38 sequenced for 40–65 residues. Only the V$_\kappa$21A subset has additional methionine residues and these fragments were isolated and sequenced. In certain cases mild acid cleavage was used to split aspartic acid–proline bonds. Arginine fragments extending over positions 65–113 were prepared for certain κ chains and sequenced for 35–40 residues. Thermolysin, tryptic and chymotryptic peptides were isolated from certain of these methionine or arginine fragments and compositions were determined. In some cases these peptides were sequenced in the presence of polybrene. A detailed analysis of these data will be presented elsewhere[28].

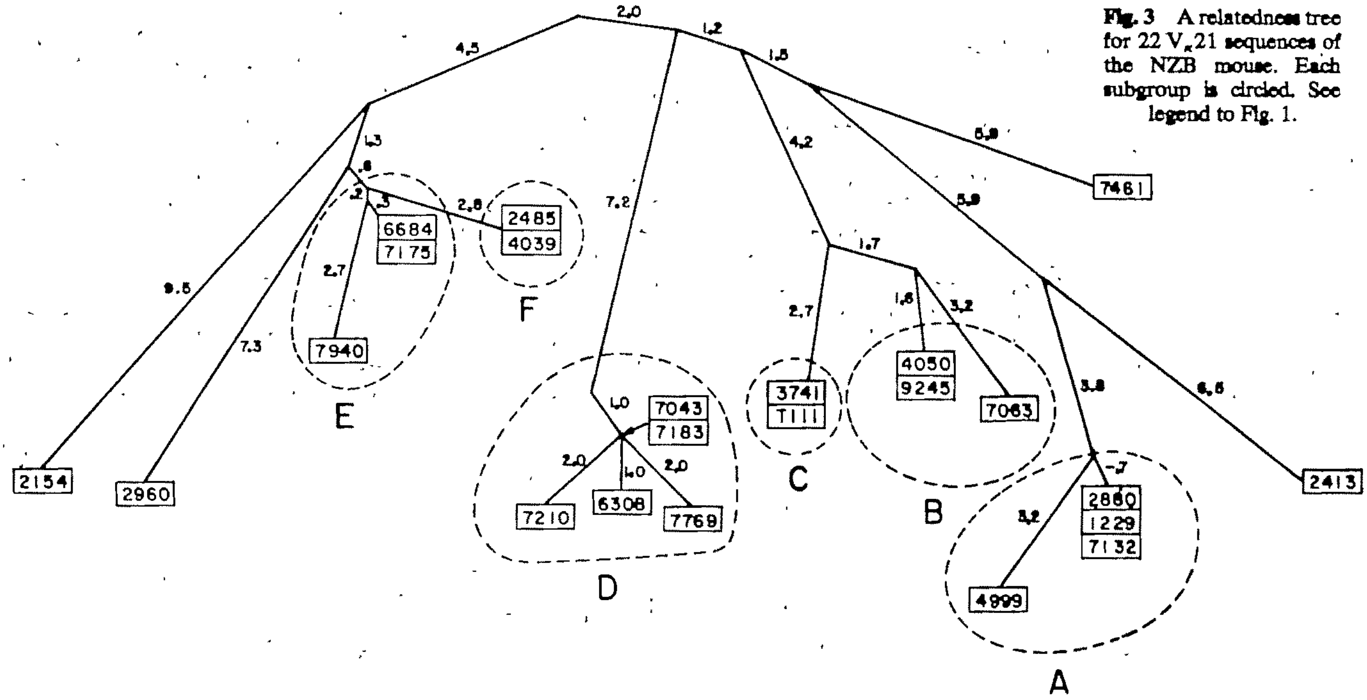


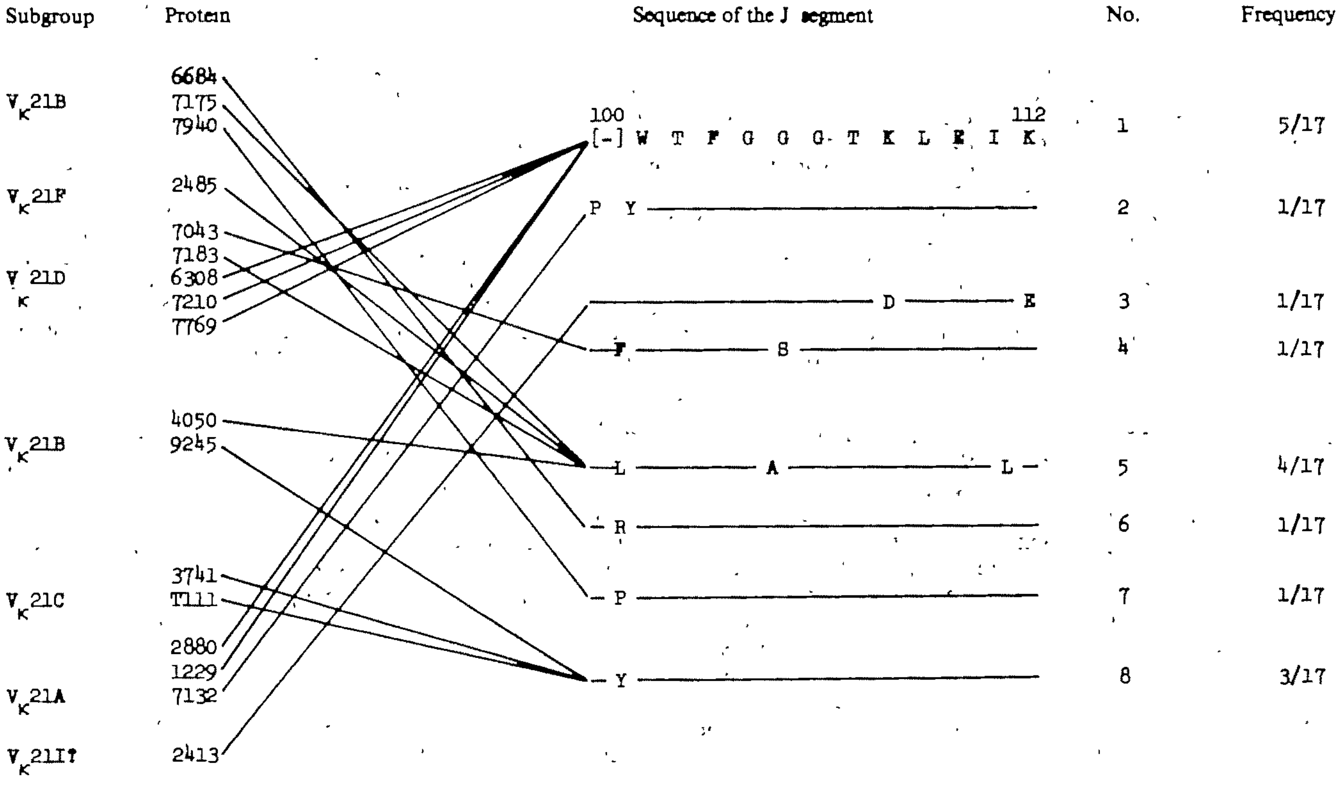
Fig. 3 A relatedness tree for 22 $V_\kappa 21$ sequences of the NZB mouse. Each subgroup is circled. See legend to Fig. 1.

single J segment may be joined in a combinatorial fashion to each V segment to provide another stage in the amplification of antibody diversity. We describe this process as combinatorial joining. Indeed, position 101 is among the most variable in the $V_\kappa 21$ regions examined (Fig. 2). This diversity might include sequence gaps (insertions or deletions) as well as nucleotide substitutions. The sequence gaps would result from J regions of different length (for example, proline at position 100 in PC7132).

The precise size of the J segment of $V_\kappa 21$ genes must wait the DNA sequence analysis of $V_\kappa 21$ genes from differentiated and undifferentiated DNA. If J does not include part of the third hypervariable region, then combinatorial joining, as such, would not generate diversity in the combining site. In this case, invariant associations such as the leucine at 101 with alanine at 105 and leucine at 111 as well as the pattern of substitutions at position 101 require an alternative explanation.

The J segment may also mediate the joining of V, J and C gene information. DNA rearrangements and mRNA splicing[36–38] must occur before the V, J and C gene information is joined into a single cytoplasmic mRNA (Fig. 5). The joining of V and J regions seems to occur at the DNA level[18]. The intervening

Fig. 4 A diagram illustrating the independent association of the V segments (residues 1–99) and the J segments (residues 100–112).



DNA sequence between the J and C regions could either lack other J regions (Fig. 5*a*) or contain them, in which case they would be eliminated by RNA processing (Fig. 5*b*).

Intra-subgroup variability

The analysis of the intra-subgroup diversity in mouse λ chains presented a simple mutational pattern consistent with somatic mutation[15]. Likewise, if each of the six $V_\kappa21$ subgroups is encoded by a single germline V gene, the nature and patterns of substitutions within each subgroup should reflect the mechanism of somatic mutation. The 12 intra-subgroup substitutions within the $V_\kappa21$ regions are indicated by circles in Fig. 2. Several features distinguish these V_κ substitutions from their V_λ counterparts discussed earlier. (1) Two of 12 substitutions are found outside the hypervariable regions. (2) Four of six variant sequences have two or more substitutions. If these differences arise by somatic mutation, the mutational mechanism operates outside as well as within hypervariable segments and shows a tendency to generate multiple substitutions in individual variant sequences. Moreover, somatic mutation followed presumably by antigen selection seems to favour diversity at certain sites in the hypervariable regions.

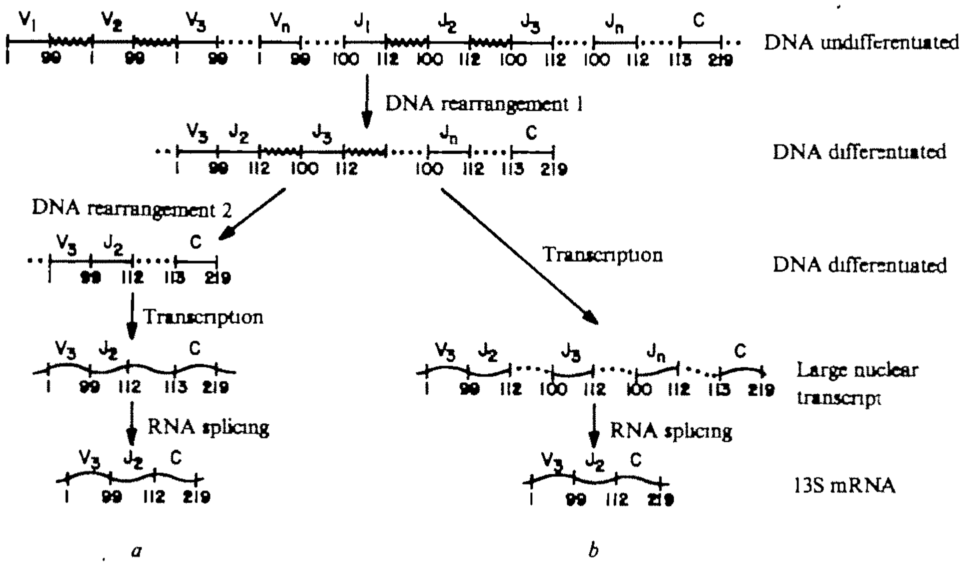


Fig. 5 Hypothetical models for the DNA segments of mouse κ genes. These gene segments are rearranged at the DNA level, presumably as the lymphocyte differentiates. As with λ genes, DNA rearrangement is depicted as joining the V and J segments[4] There are at least two pathways for joining the J and C segments at the nucleotide level. *a*, A second DNA rearrangement moves the J_2 segment closer to the C segment, possibly deleting the intervening J sequences. A recent report suggests that two or more DNA rearrangement events can occur in lymphocytes during the switch in synthesis from one to a different immunoglobulin class[48]. Final joining occurs by mRNA splicing. *b*, A large nuclear RNA transcript complete with the intervening J segments is processed by mRNA splicing enzymes

Again, this analysis of intra-subgroup variability must be qualified by the possibility that one variant differing by two or more residues from the prototype sequence will become a distinct subgroup when a second identical sequence is determined. Thus, perhaps the reason the intra-subgroup variability in the $V_\kappa21$ chains and the λ chains differ is that in the former some of the variants are encoded by additional germline V genes.

Sources of antibody diversity

The analysis of the V_κ 21 group of mouse V_κ regions offers certain insights into each of four basic mechanisms for producing antibody diversity.

Multiple germline V genes: According to a statistical analysis of N-terminal data, there are about 50 κ groups[15,39]. This number may be an underestimate because it is based on the sequences of myeloma proteins which may represent only a subset of the total V genes. Our studies suggest the $V_\kappa21$ group is encoded by at least six V genes. In addition, studies at the DNA level on three V_κ groups suggest that four to eight germline V genes are present in each group[23,32]. If we assume that seven germline V genes are present in each group defined by N-terminal analysis, then approximately 350 germline genes encode the V_κ family. Less is known about the diversity in the V_H gene family, but a similar estimate of at least 200 V_H genes seems reasonable.[39] In contrast, the mouse λ gene family is apparently encoded by one or two germline genes.

Combinatorial association: That most light chains may combine with most heavy chains is suggested by several types of observations[19,40,41]. This non-genetic mechanism for generating antibody diversity is termed combinatorial association. In principle, 350 V_L and 200 V_H regions could, through combinatorial association, generate 7×10^4 different types of antibody molecules. If the mammalian antibody repertoire is greater than 10^6 (see ref. 42), and if we assume that there are only 350 V_L and 200 V_H germline genes, then more than 90% of the antibody repertoire must be generated by somatic mechanisms.

Somatic mutation: All the intra-subgroup substitutions noted in the $V_\kappa21$ regions are single-base substitutions, predominantly in the hypervariable regions. Obviously, some or all of this variation could arise by spontaneous somatic mutation or by a hypermutational mechanism.

Combinatorial joining: One potential source of somatic diversification is the combinatorial joining of multiple J segments and V segments (Fig. 5). Thirteen different J segments have already been identified in mouse κ chains. For example, if 10 different J segments may be joined to any of 350 different V segments, then 3,500 different V regions may be generated. If combinatorial joining of a similar magnitude occurs in light and heavy variable regions, 7×10^6 antibody molecules could be generated by this process.

Combinatorial joining could generate identical variants among mice of the same inbred strain In contrast, most somatic theories have assumed that the generation of diversity is a random process occurring by mutation, recombination or gene conversion. A reproducible somatic process could explain the patterns of diversity seen in certain immune responses. For example, the responses to α1,3-glucosyl linkages of BALB/c mice[43] and to the 4-hydroxy-3-nitrophenylacetyl hapten (NP) of C57BL/6 mice[44] are heterogeneous by isoelectric focusing, but the heterogeneity is limited to V_H and is reproducible in each case[45,46]. Perhaps this reproducible heterogeneity arises from the combinatorial joining of V_H–DEX or V_H–NP^b genes with a variety of different J_H segments. A similar possibility could explain the closely related heavy-chain sequences produced in response to the immunisation of guinea pigs against several haptens[47].

Other investigators have proposed combinatorial joining as a mechanism for antibody variability[7,8], but their models arose from the conviction that certain hypervariable regions may be associated with many different framework regions. For example, based on a computer analysis of all available V-region sequences, Kabat *et al.* have suggested the mini-gene model, which proposes that each of the three hypervariable regions and the four intervening framework regions are encoded by minigenes which are joined during differentiation[9]. The correlation of specific hypervariable and framework segments within certain V_κ21 subgroups (Fig. 2) argue against this general model. Our data suggest that Kabat's model is correct in principle, but that the combinatorial process occurs only within the third hypervariable region. The identification of a J segment in λ chains raises the possibility that combinatorial joining of V and J segments will be used by all three antibody families, λ, κ and H.

Conclusion

Antibody variability may be generated by a variety of mechanisms, some genetic (multiple V genes, combinatorial joining and somatic mutation) and others non-genetic (combinatorial association). Combinatorial joining is both a somatic and a germline mechanism in that it presumably rearranges germline information during somatic differentiation. The critical question that remains for the future is what are the relative contributions of these four basic mechanisms for generating diversity to the functional repertoire of antibody molecules? The answer to this intriguing question will obviously require combined chemical, biological and genetical approaches.

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The arrangement and rearrangement of antibody genes

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Cloned segments of mouse chromosomal DNA provide direct evidence for the somatic rearrangement of κ variable and constant region genes in antibody producing cells. This rearrangement apparently affects only one member of an allelic pair of light chain genes.

THE notion that immunoglobulin genes are encoded in several segments that rearrange during somatic differentiation has been incorporated into most theories of antibody diversity since the 'two gene–one polypeptide' argument was first advanced by Dryer and Bennet in 1965 (ref. 1). Most recently, certain details of this arrangement have become clear through a series of experiments involving the analysis of fragments containing chromosomal DNA encoding variable and constant region immunoglubulin genes. As predicted, constant and variable regions are separately encoded in germline DNA[2–4] and appear to be brought together by a somatic recombination event[5–7]. Moreover, it seems likely that this recombination is closely and critically related to light chain production; that is, that the rearranged variable region sequence will be the one expressed in a given immunocyte.

Further analysis of these genes, several of which have been cloned, indicates that their organisation is even more complex than originally suspected. The inactive κ and λ mouse variable region genes sequenced thus far fail to encode the last 10 or so amino acids that had, by sequence analysis, always been associated with the variable region[4,8]. In a direct examination of a

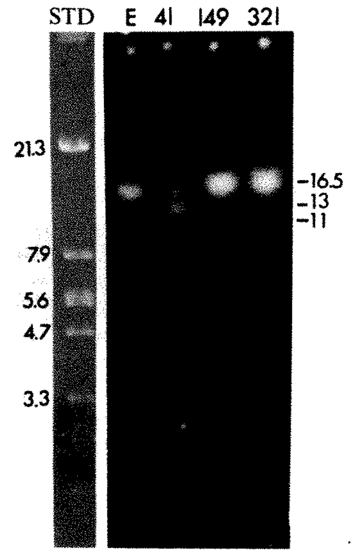

Fig. 1 Analysis of the *Bam*H1 fragments of BALB/c embryonic and plasmacytoma DNAs annealed to a constant region gene sequence. The fragments obtaind by BamH1 digestion of embryonic (E), MOPC-41 (41), MOPC-149 (149) and MOPC-321 (321) DNA were fractionated by electrophoresis (from top to bottom) in a 0.7% agarose gel, denatured, neutralised, transferred to nitrocellulose sheets and hybridised with a ^{32}P-labelled DNA fragment (136 c.p.m. per pg) which contained constant region sequences as described previously[4]. The constant region containing DNA fragment used as probe was the 2.8-kilobase *Bam*H1–*Hin*dIII fragment indicated in Fig. 6, which contains the κ constant region gene. The embryo specific band (16.5 kilobase) and the plasmacytoma specific bands (13.0 and 11.0 kilobases) are indicated. Fragment lengths were determined by comparison with ethidium bromide-stained, *Eco*R1 fragments of phage λ DNA of indicated length (STD).

cloned embryonic mouse λ light chain DNA fragment, Tonegawa and his associates have shown that this terminal variable region segment, called the J sequence, is separately encoded[9]. While the precise borders of these regions have not yet been determined, it seems that some—if not all—light chain genes are encoded in at least three segments, a major variable region sequence, a short J sequence and the constant region sequence. This observation also seems to fit in with recent comparisons of amino acid sequence data (ref. 10 and M. G. Weigert and L. Hood, personal communication).

Since the somatic rearrangement of these three segments may play a part in generating antibody diversity as well as in antibody gene activation, it will be important to determine whether gene rearrangement is a constant feature of immunocyte development and to understand its precise molecular basis. In some respects this is most advantageously investigated in the mouse κ light chain system where hundreds of variable region genes exist in the embryonic chromosome[4,11] and are presumably candidates for rearrangement and, hence, expression.

In what follows we describe the cloning and characteristics of several immunoglobulin κ light chain genes derived from the chromosomes of embryonic and κ chain producing cells. While different gene arrangements appear in each producing myeloma cell line, the basic embryonic gene arrangement is also preserved in each. One of the rearranged fragments contains an entire light chain sequence in which variable and J sequences appear contiguous and are separated from the constant region gene by a 3.7-kilobase intervening sequence of DNA. Several cloned κ gene fragments have also recently been obtained by Lennard-Schuller *et al.*[21].

Cloning fragments containing constant region genes

By using hybridisation probes derived from cloned κ light chain cDNA, we were previously able to identify several *Eco*R1 fragments of embryonic and myeloma DNA (MOPC-149 and MOPC-41) that encoded closely related variable region gene sequences[11]. In contrast, each tissue yielded what appeared to be a single *Eco*R1 fragment of DNA encoding a constant region sequence that curiously migrated at approximately the same position in a two-dimensional analysis involving RPC-5 chromatography and gel electrophoresis[11]. Evidently, whatever rearrangement occurred amongst these genes, it resulted in the production of new DNA sequences that closely resembled the embryonic *Eco*R1 pattern in length and chromatographic properties.

That the gene rearrangement had actually occurred in MOPC-149 and MOPC-41 cells could be demonstrated using a different restriction enzyme to produce a new fragment pattern in the producing cells. DNA from embryonic, MOPC-41, MOPC-149 and MOPC-321 cells were digested with *Bam*H1, electrophoresed, blotted to nitrocellulose sheets[12] and annealed to a radioactive probe corresponding to the κ constant region (Fig. 1). Each tissue, including embryo, yielded a 16.5-kilobase DNA fragment that contained constant region sequences. Each myeloma, on the other hand, yielded an additional fragment, in the case of MOPC-41, approximately 13 kilobases long, in the case of MOPC-149 and MOPC-321, 11 kilobases long. (MOPC-321 was previously analysed with *Bam*H1 by Hozumi and Tonegawa[5] and was included as a control. The constant fragments we observe differ in size from those seen by the earlier workers and retain the germline fragment which was apparently not observed in their analysis. It is possible that we are dealing with different tumours.) Given these results, it seemed likely

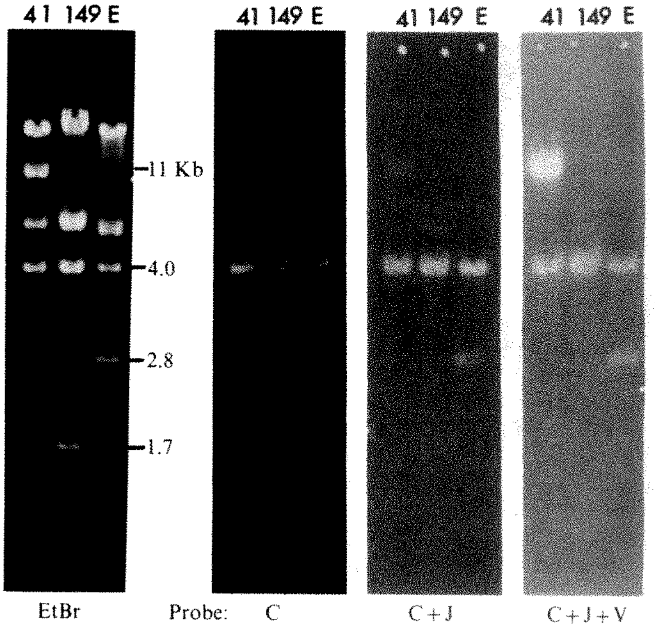

Fig. 2 Restriction endonuclease *Hin*dIII digestion of cloned embryonic and plasmacytoma DNA fragments encoding κ gene sequences. The fragments obtained by *Hin*dIII digestion of λCh4A phage containing 41KC-21, 149C-2 and EC-5 *Eco*R1 DNA fragments were fractionated on a 1.2% agarose gel and stained with ethidium bromide (EtBr). The *Hin*dIII fragments (left) were denatured, transferred to nitrocellulose sheets and hybridised separately to three ^{32}P-labelled probes and autoradiographed (right) as described previously[4,11]. The probes were ^{32}P-labelled fragments containing segments of the κ light chain. The constant region probe was a *Hpa*I/*Bam*H1 fragment obtained from pBR322-K41 (ref. 11) which should encode from amino acids 125 through the 3' untranslated end. The J region (J+C) probe was a *Hin*cII fragment obtained from pCR1-K149 (ref. 19) which encodes amino acids 90–125 of the MOPC-149 light chain. The variable region probe ($V+J+C$) was a *Bam*H1/*Hin*cII fragment cleaved out of pBR322-K41 (ref. 11) which encodes amino acids 44–125 of the MOPC-41 light chain. The sizes in kilobases of the *Hin*dII fragments were determined relative to phage λ *Eco*R1 fragments. 149KC-2 does not have a separate fragment that hybridises to the J region probe, while 41KC-21 has both a J region and a variable region gene sequence.

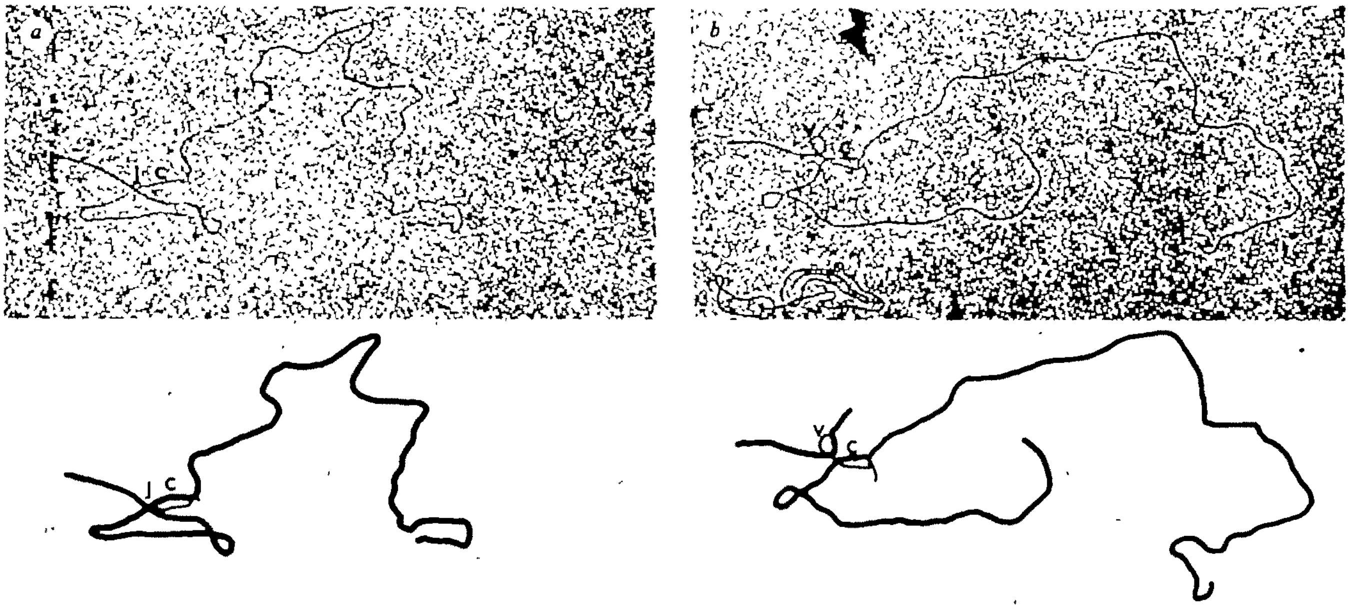

Fig. 3 Visualisation of the κ light chain sequences encoded by two cloned *Eco*R1 fragments derived from embryonic DNA and MOPC-149 DNA. *a*, The 16.0-kilobase *Eco*R1 fragment isolated from the phage λCh4A-EC-5 was annealed to MOPC-41 mRNA and spread for electron microscopic analysis as described previously[21]. *b*, The 14.9-kilobase *Eco*R1 fragment (20 μg ml^{-1}) from the phage λCh4A-149C-2 and an 1800-base pair *Hae*III fragment (20 μg ml^{-1}), from pMB9-K2 which encodes a MOPC-149-like variable region[4] were annealed with a MOPC-149 mRNA (10 μg ml^{-1}) in 70% formamide, 0.5 M NaCl, 0.1 M Tricine *p*H 8.0, 0.01 M EDTA at 52°C for 18 h, and spread for electron microscopy. The R loops are interpreted by diagrams drawn immediately below each molecule. The constant region R loop (C), 475 ± 78 base pairs long and the variable region R loop (V) is 299 ± 68 base pairs long. The analysis of 35 molecules suggested that the constant region R-loop is 10.4 ± 0.24 kilobases, from one end and 5.1 ± 0.20 kilobases from the other end of the fragment. The location of the *J* region gene was determined by the distance, 1.3 ± 0.19 kilobases, from the end of the fragment to the point where the DNA falls across the end of the R loop. The long ends of both DNA fragments are single stranded because of nicks introduced into the DNA by nuclease in the *Eco*R1 preparation Although some of the *Eco*R1 fragments had single stranded ends nearly all of the fragments were full length when the single strand DNA was included in the length of the fragment.

that the *Eco*R1 digestion of the DNA from each tumour produced a mixture of germline and rearranged fragments of approximately the same size. Obviously their structural features could best be resolved by cloning.

The *Eco*R1 fragments from embryonic and plasmacytoma DNA which contained constant region gene sequences were therefore cloned, using procedures previously used to clone the mouse globin[13–15] and κ variable region genes[4]. *Eco*R1 fragments containing κ constant region sequences were purified first by RPC-5 column chromatography and then by preparative gel electrophoresis, ligated to the DNA of the EK2 vector λCh4A (ref. 16) and finally packaged into phage particles using the procedure developed by Becker and Gould[17] and Sternberg, Tiemeier and Enquist[18] as modified by F. Blattner and his co-workers (personal communication). In this way, 8×10^4 hybrid phage were derived carrying embryonic DNA fragments. Of these, 20 contained constant region sequences. Twenty thousand hybrid phage were derived carrying MOPC-149 DNA fragments. Four contained constant region sequences. Ten thousand hybrid phage were derived carrying MOPC-41 DNA fragments. Three contained constant region sequences.

Light chain producing cells contain both embryonic and rearranged constant region genes: restriction enzyme mapping

Antibody production seems to involve the expression of only a single allele of the pair of genes presumably present in diploid cells. While various potential mechanisms might be invoked to account for this allelic exclusion, it is appealing to consider that the rearrangement of only one member of an allelic pair might create only one complete and functional copy of light and heavy chain genes. We have already noted that the constant region *Bam*H1 restriction fragment pattern derived from the plasmacytoma cells reveals one fragment that is the same size as that seen in embryonic DNA (Fig. 1) If only one of the constant region alleles has undergone rearrangement, we might expect to find, in addition to the rearranged fragment, several fragments among our plasmacytoma-derived clones that are identical to those cloned from embryonic DNA, that is, non-rearranged fragments. To test this possibility, the DNA from five of the embryo-derived constant region gene containing phage and DNA from each of the phage carrying MOPC-41 and MOPC-149 constant region sequences were cleaved with several restriction enzymes and the resulting fragments were fractionated on agarose gels (data not shown). Not surprisingly, all five phage DNAs isolated from embryonic DNA were apparently identical (except with respect to their orientation within the vector). Furthermore, three of the four MOPC-149 derived phage and two of the three MOPC-41 derived phage seemed to be identical to the phage derived from embryonic DNA. It is possible that some of these embryo-like fragments were derived from non-plasmacytoma cells present in tumour tissue. This seems less likely in view of the minor degree of tissue contamination observed microscopically and the prevalence of embryo-like fragments among hybrid clones.

Although several of the cloned fragments obtained from plasmacytoma DNA were apparently identical to that derived from embryonic DNA, one cloned fragment from each light chain producing cell differed from the embryonic DNA fragment. These two isolates, derived from plasmacytoma MOPC-149 and MOPC-41 DNAs, were designated 149KC-2 and 41KC-21, respectively. (The phage isolate containing the embryo-derived *Eco*R1 fragment which was used for comparison in these studies was called EC-5.) When the phage DNAs were cleaved with *Eco*R1 and fractionated on a 0.6% agarose gel, small differences in the sizes of the three constant region containing fragments could be detected; the fragments EC-5, 149KC-2 and 41KC-21 were 16.0, 14.9 and 16.5 kilobases respectively (data not shown). While these differences became more obvious when the hybrid phage were cleaved with the restriction endonuclease *Hin*dIII (Fig. 2), the differences between the fragments were not great, suggesting that there was substantial homology between them. Indeed, most enzyme digests produced several fragments that were identical in length among the three phage and yielded only one or two fragments that actually differed.

To determine which of the restriction fragments contain portions of the light chain structural gene, the separated fragments were denatured in the gel, transferred to nitrocellulose sheets and hybridised to the appropriate ^{32}P-labelled probes corresponding to different segments of the κ light chain mRNA. An example of results obtained in this way is shown in Fig. 2. A single 4.0-kilobase *Hin*dIII fragment anneals to a cloned constant region cDNA probe (extending from codon position 125 through the 3′ untranslated sequence) and is found in all three genomic fragments. Surprisingly, a probe incorporating the variable–constant region junction (codon 90–125), presumably including the suspected J sequence, hybridised to two fragments in the digest of the embryonic clone, indicating that this sequence is interrupted by *Hin*dIII site in the embryonic genome. The second fragment is absent from MOPC-149 clone, but is present on the clone derived from MOPC-41 DNA (Fig. 2). Apparently, this particular J region gene sequence has been lost in the chromosomal rearrangement that produced the new MOPC-149 fragment.

Since two of these cloned *Eco*R1 fragments (from embryo and MOPC-41 DNA) contained both a J region gene and a constant region gene, either might also carry a variable region gene. The fragment derived from MOPC-149 DNA, while rearranged, failed to hybridise to MOPC-149 variable region coding sequence (data not shown). Therefore, as we had already concluded from previous analyses of genomic DNA[4], an *Eco*R1 site separates variable and constant region genes in the MOPC-149 genome. On the other hand, the MOPC-41 V + J + C region gene probe hybridises much more strongly the 11.0-kilobase *Hin*dIII fragment derived from the MOPC-41 hybrid phage than did the probe containing only the J and constant region sequences. This suggests that the new MOPC-41 *Eco*R1 fragment might contain the variable region as well as the J and the constant region sequences (see below). That is, the entire MOPC-41 light chain structural gene might be encoded in this single *Eco*R1 fragment.

Visualisation of embryonic and rearranged MOPC-149 gene sequences

In situ hybridisation analysis indicated that the three *Eco*R1 constant region fragments contained extensive regions of homology, but were nevertheless different from one another. The embryonic clone—which was repeatedly discovered among cloned fragments derived from each plasmacytoma—encoded a constant region sequence and a J sequence homologous to that represented in MOPC-41 cDNA. The rearranged DNA segment derived from MOPC-149 DNA encoded a constant region sequence, but had evidently lost the nearby J region segment and, as a new *Eco*R1 site interceded between constant and variable region, a MOPC-149 variable region sequence did not occur on our cloned fragment. The rearranged DNA segment derived from the MOPC-41 DNA, on the other hand, seemed to encode all three segments. (These features are summarised in the map of each fragment represented in Fig. 6.)

The presence of each of these structural features could be confirmed and their relative position on each fragment determined by direct visualisation of R-loop structures formed by annealing light chain (MOPC-149 or MOPC-41) mRNA to each segment. In appropriate conditions, the light chain mRNA anneals to complementary sequences in one of the DNA strands forming a double-stranded RNA–DNA hybrid and displacing the other strand to form a bubble-like structure called an R loop[20]. When the embryonic fragment (EC-5) was examined in this way, a single R loop 475 ± 78 base pairs long was observed, located 5.1 ± 0.20 and 10.4 ± 0.24 kilobases from the ends of the fragment (Fig. 3*a*). A similar structure was observed when the fragment derived from MOPC-149 was analysed (Fig. 3*b*). In both cases, the loop structure should represent the constant region sequence (Fig. 2). This could be confirmed and the gene orientated (5′ – 3′) by forming a second R loop using a previously cloned DNA fragment known to encode a MOPC-149-like variable region gene[4] (Fig. 3*b*). Here the light chain mRNA links the two cloned fragments by forming an R loop on the smaller variable region fragment (1.8-kilobase *Hae*III fragment of pMB9-K2)[4] and the larger constant region fragment. The smaller fragment serves as a marker to identify the 5′ side of the constant region R loop and confirms that the constant region sequence is annealed to the larger, MOPC-149 fragment. Similar structures were visualised using the embryonic fragment.

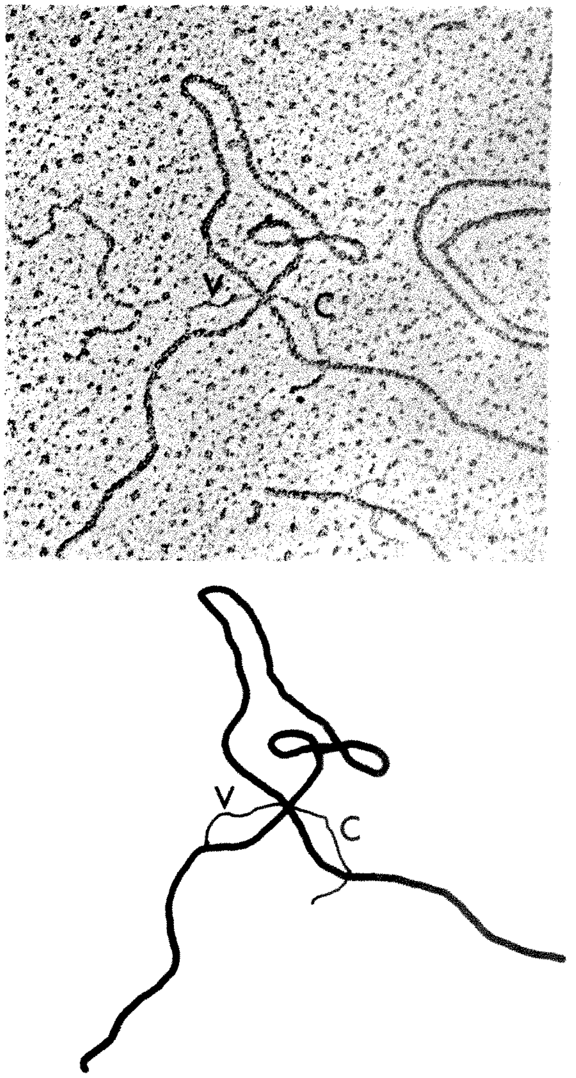

Fig. 4 The R loop formed between a fragment cloned from MOPC-41 DNA and MOPC-41 mRNA. The variable (V) and constant (C) region R loops formed as described previously[4] are separated by 3.7 ± 0.187 kilobases of DNA. Twenty-five molecules were measured.

The J region sequence on the embryonic fragment could also be identified by electron microscopic visualisation, despite its small size. The J region is too small to form a visible R loop, but could form a stable hybrid when annealed to MOPC-149 mRNA (Fig. 3*a*). Our hybridisation studies already suggested that this sequence was not immediately adjacent to the constant region sequence (Fig. 2). Indeed, if the J region were encoded at a distance from the constant region gene, we would expect to find molecules containing a constant region R loop and an intervening loop of double-stranded DNA joined to the constant region R loop by the J sequence in the mRNA. We identified 10 such structures in which a ~3.7-kilobase length of

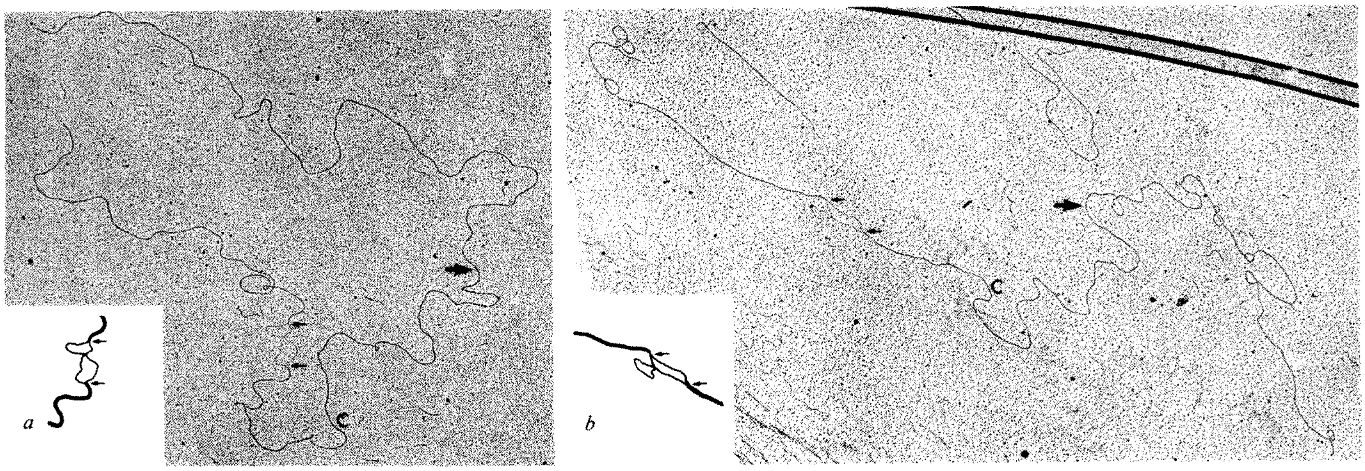

Fig. 5 Heteroduplexes formed between DNAs carrying embryonic and plasmacytoma constant region gene sequences. *a*, A heteroduplex formed between λCh4A-EC-5 (embryonic) and λCh4A-41C-21 (plasmacytoma) DNA. *b*, A heteroduplex formed between Ch4A-EC-5 and Ch4A-149C-2 (plasmacytoma) DNA. The techniques for forming the heteroduplexes and spreading them for electron microscopy were as before[14]. The large arrow indicates the approximate location of the *Eco*R1 site in the phage DNA which is about 10.4 kilobases from the constant region gene (indicated by 'C'). The smaller arrows indicate the ends of single stranded regions which indicate non-homology between the two DNAs. The non-homology seen in these heteroduplexes is due to a DNA sequence present in plasmacytoma DNA, presumably introduced by a recombination event that occurred during the differentiation of the MOPC-149 and MOPC-41 cell lines. The measured lengths of single-stranded DNA in these heteroduplexes was used to determine the exact location of the recombination event with respect to ends of the *Eco*R1 fragments. Twenty-five molecules were measured.

DNA recrossed itself adjacent to the 5′ end of the constant region R loop as shown by the molecule in Fig. 3*a*. That this structure is merely the result of the DNA twisting back over itself seems unlikely for three reasons. First, we never saw the DNA from the 10.4-kilobase side of the fragment twist over the end of the R loop. Second, in the 10 molecules observed, the J sequence was always 1.3±0.19 kilobases from the end of the fragment. Third, we could not find this hairpin structure when we formed an R loop between MOPC-149 mRNA and the MOPC-149 DNA fragment and we knew from *in situ* hybridisation studies (Fig. 2) that this fragment does not have a J region sequence (Fig. 2). Thus, we conclude that the embryonic *Eco*R1 fragment contains a J region gene about 3.7 kilobases from the constant region gene. These features are summarised diagrammatically in Fig. 6.

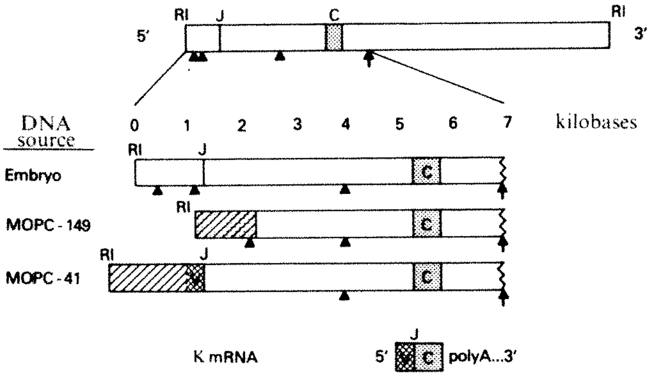

Fig. 6 Arrangement of light chain gene sequences on the *Eco*R1 fragments cloned from embryonic and plasmacytoma DNA. The *Eco*R1 fragment from embryo (16.0 kilobase) DNA is shown with the 5′ end of the constant region gene to the left. The portions of the cloned fragments which differed from one another are shown on an expanded scale. The positions of the *Eco*R1, *Bam*H1 and *Hin*dIII restriction sites in this region are indicated. The segments of the light chain, the variable region (V), the J sequence (J) and the constant region (C) are indicated. The hatched segments in the plasmacytoma fragments, MOPC-149 and MOPC-41, represent DNA segments that are not present in the embryonic fragment. Note that they are different from each other as well. The κ light chain mRNA is diagrammed to indicate the size of the structural gene relative to the size of the intervening sequence in the MOPC-41 light chain gene.

The rearranged MOPC-41 fragment encodes a complete light chain gene and is interrupted by a 3.7-kilobase intervening sequence

In contrast to the embryonic and MOPC-149 segments, the 16.5-kilobase *Eco*R1 fragment cloned from MOPC-41 DNA hybridised to all three segments of the light chain sequence (Fig. 2). The R loop structure formed by annealing this fragment to MOPC-41 mRNA reflects this difference (Fig. 4). Instead of a single R loop, two are formed. Both flank a large, 3.7-kilobase intervening sequence that interrupts the structural gene and is not represented in the mature light chain mRNA. A tail, probably representing poly A, is frequently seen on the constant region end of the structure and its associated R loop measures 409±128 base pairs. The variable region R loop measures 229±68 base pairs. The sequence is also represented diagrammatically in Fig. 6.

Recombination sites defined on the two plasmacytoma-derived *Eco*R1 fragments

We have shown by restriction mapping and R loop analyses that the two plasmacytoma-derived fragments are related to, but different from each other and the fragment derived from embryonic DNA. In order to define more closely the relationship between these three constant region encoding fragments and to compare their sequences, we have formed heteroduplexes between these fragments cloned in the λCH4A vector. An example of the heteroduplex structures formed between the phage containing the embryonic fragment and the phage containing the MOPC-41 segment is shown in Fig. 5*a*. The genomic fragments (whose ends are defined by the arrows closest to the ends of the double-stranded DNA in Fig. 5*a*) form a homologous, double-stranded structure for about 14.8 kilobases and then diverge at one end (the single-stranded bubble). Since the right arm of the λCh4A vector DNA is shorter than the left[16], the orientation of the fragment and the location of the differences between the two fragments are defined at the points of divergence in the heteroduplex molecules. Thus, the rearranged MOPC-41 fragment differs from embryonic fragments by a segment of DNA introduced at its 5′ end at approximately the position of the embryonic J sequence (Fig. 6).

Similarly, the point of divergence in the rearranged MOPC-149 fragment could be determined. A heteroduplex formed between the phages containing the embryonic fragment and the

MOPC-149 fragment is shown in Fig. 5*b*. The fragments are homologous for 13.5 kilobases, but also diverge at their 5′ end. In each case, the divergent heteroduplex bubble represents DNA that is different in the embryonic sequence. Evidently these new sequences are derived from a recombination-like event which joined DNA sequences to the 5′ ends of plasmacytoma DNA fragments and eliminated sequences present in the embryonic fragment (see diagram, Fig. 6). By measuring the lengths of the single-stranded DNAs found in each heteroduplex, we can estimate the lengths of the new DNA sequences that have been joined to the embryonic *Eco*R1 fragment. The points at which the embryonic fragment differs from each rearranged fragment defines the site at which the recombination event took place.

The recombination events that formed the new MOPC-149 and MOPC-41 fragments involved different sites. In MOPC-149 the recombination event appears to have occurred 2.7 kilobases from the constant region gene and removed the MOPC-41-like J region sequence from the embryonic fragment. Presumably the MOPC-149 light chain uses a different J region sequence. The recombination event that generated the MOPC-41 light chain sequence occurred 3.7 kilobases from the constant region gene, very close to the J region sequence found in the embryonic fragment. Here a single recombination event may have linked the MOPC-41 variable region to the MOPC-41 J region to generate the cloned fragment. However, until the nucleotide seuences of the appropriate regions have been determined, we cannot be certain that the recombination event that generated the MOPC-41 gene did not actually replace the embryonic J region with a MOPC-41 J region at the same relative position. It is also possible that both J region sequences are present on the MOPC-41-derived fragment. Nevertheless, these analyses indicate that the recombination events that affect immunoglobulin light chain genes can involve sites at least 1.0 kilobases apart.

The emerging picture of antibody gene arrangement and rearrangement

By characterising cloned, κ chain-encoding segments of DNA derived from mouse embryo and antibody producing cells, we demonstrate directly that immunoglobulin constant and variable region genes undergo a somatic rearrangement that brings together the segments necessary to encode a complete κ light chain gene. Moreover, in each light chain producing plasmacytoma that we have thus far examined there is evidence of somatic rearrangement of one set of these genes, while a second, presumably allelic, set retains the original embryonic pattern. These observations fit well with the plausible notion that rearrangement of a single allele is instrumental in immunoglobulin gene activation and that it may play a part in allelic exclusion in antibody production.

In addition we see that, like the mouse λ light chain[9], κ genes are encoded in at least three segments in embryonic DNA, a foreshortened variable region sequence[4], a short J sequence probably encoding the last 10 or so amino acids of the variable region and, finally, the constant region sequence. Rearrangement of these sequences (which may itself contribute to the generation of antibody diversity) forms, in the case of the MOPC-41 light chain gene, a new chromosomal sequence in which variable and J sequences are adjacent, but separated from the constant region gene by a large 3.7-kilobase intervening sequence of DNA. In the case of MOPC-149, this rearrangement has eliminated the embryonic J sequence and replaced it, in our clone, with a new segment of DNA. Whether this new segment is a portion of the intervening sequence linking MOPC-149 variable and constant regions in genomic DNA is not known. In contrast to what might have been expected, these rearrangements do not occur at a single site, but the new DNA segments are joined at sites about 1.0 kilobases apart on the 5′ side of the constant region gene. The precise nature and the molecular basis of this recombination remains unclear. Further studies should indicate whether or not reciprocal recombination is involved and whether this recombination is mediated by regions of sequence homology.

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Synthesis of growth hormone by bacteria

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A hybrid gene was constructed between the β-lactamase gene of plasmid pBR322 and the cloned coding sequence for rat growth hormone. This gene is expressed in bacteria and growth hormone sequences are detectable by immunological methods.

RECENT advances in recombinant DNA technology have led to the cloning in bacteria of the natural coding sequences for several mammalian peptide hormones[1–3]. These sequences provide excellent material for testing the feasibility of producing mammalian proteins in bacteria. The success of such production depends on the correct design and construction of new genes in which suitable bacterial control elements promote expression of

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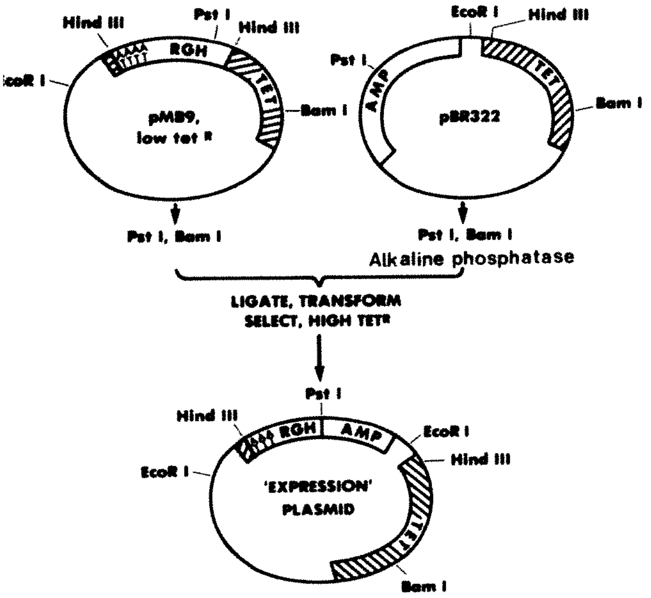

Fig. 1 Construction of a vector for bacterial synthesis of GH sequences. The cloned RGH cDNA was cleaved from plasmid pRGH-1 (ref. 2) with *Hin*dIII endonuclease and isolated by polyacrylamide gel electrophoresis. This DNA was enzymatically ligated to similarly cleaved and alkaline phosphatase-treated[1,3] pMB9. The ligation mixture was used to transform *E. coli* strain χ1776 (refs 1–3). Colonies containing plasmids were selected by growth on low levels (5 μg ml^{-1}) of tetracycline[11]. Resistant colonies were examined for the presence of plasmids containing inserted RGH sequences by gel analysis of such plasmids after digestion with restriction endonuclease *Pst*I and *Eco*RI. In some plasmids RGH DNA was found to have its poly dA · dT end orientated towards the single *Eco*RI site in pMB9. Such a plasmid (pMB9-RGH) was used in the subsequent steps for vector construction. The pre-RGH sequences from pMB9 were placed under the control of the β-lactamase gene of pBR322, as indicated. Plasmid pMB9-RGH was digested with *Pst*I plus *Bam*HI endonuclease. The single cleavage site for *Pst*I in this plasmid occurs between amino acids −24 and −23 of the pre-sequence of RGH (see Fig. 2), whereas the single *Bam*HI site is in the gene responsible for bacterial tetracycline resistance[9]. The amp^r and tet^r plasmid pBR322 was similarly digested with these two enzymes. In pBR322 the *Pst*I site occurs at a sequence coding for amino acids 182 and 183 of pre-β-lactamase[10]. The *Bam*HI site is in the *tet*^{r} gene and is in exactly the same place as in pMB9, as this region of pBR322 was derived from pMB9 (ref. 9). After digestion, pBR322 was treated with bacterial alkaline phosphatase[1,3]. This mixture was then ligated to the *Pst*I and *Bam*HI cleaved pMB9-RGH. After transformation of *E. coli*, colonies were selected for their ability to grow on high levels (20 μg ml^{-1}) of tetracycline.

the appropriate coding sequences. We describe here the construction of such a hybrid gene on a bacterial plasmid designed to program bacteria for the synthesis of pituitary growth hormone (GH) sequences. This peptide hormone is crucial for linear growth. A sufficient supply would allow wider therapeutic use in the treatment of growth disorders[4–6] and other conditions in man and might be useful in animal husbandry for more economical food production. The species specificity and size (190 amino acids) of GH[7,8] prevents us from exploiting existing resources or applying current synthetic techniques. Thus, for GH and similarly for a variety of other eukaryotic proteins, genetically programmed bacteria may provide an important resource.

Gene construction

Table 1 Radioimmunoassay of RGH in extracts from minicells containing pEx-RGH or pBR322

Plasmid	RGH by radioimmunoassay (ng ml^{-1})	
	Spheroplasts	Periplasmic space
pEx-RGH	89.1 (70.8–116.6)	30.8 (30.0, 31.6)
pBR322	Undetectable	Undetectable

One litre of *E. coli* P648-54 containing either pEx-RGH or pBR322 was grown to saturation and collected by centrifugation at 10,000*g* for 10 min. The cells were washed and spheroplasts were prepared by modification of procedures described elsewhere[15]. Briefly, cells were washed once in 200 ml of 10 mM Tris (*p*H 7.4) containing 1 mM PMSF (phenylmethylsulphonyl fluoride, Sigma). The cells were resuspended in 50 ml of 20% sucrose, 0.033 Tris (*p*H 8.0) and 1 mM PMSF. Ice-cold 0.1 M EDTA (*p*H 8.0) was added (0.5 ml) and the mixtures were kept on ice for 5 min. After addition of 1 ml of 5 mg ml^{-1} lysozyme (Sigma) the mixtures were incubated for 30 min at 0 °C and centrifuged (17,000*g*, 10 min, 0 °C). The supernatant media were saved for radioimmunoassay of material from the periplasmic space. The pellets were resuspended in 10 mM Tris (*p*H 8.0) and 1 mM PMSF. $MgCl_2$ was added to a final concentration of 10 mM with 200 μl each of DNase (1 mg ml^{-1}) and RNase (5 mg ml^{-1}). Incubation proceeded for 1 h at 4 °C (spheroplast sample). These mixtures (10 ml) and 50 ml of the samples containing periplasmic material were precipitated with $(NH_4)_2SO_4$ (50% saturation). The samples were resuspended in 4 ml of 50 mM Tris (*p*H 9.0), 0.1 M NaCl and 1 mM PMSF and dialysed against 2 l of the same buffer for 2 h at 4 °C. Serial dilutions of these samples were analysed by radioimmunoassay as described previously[19]. Amounts of RGH found by this assay are listed in ng ml^{-1} with the mean and range of these determinations for the spheroplast sample and the mean and individual values for duplicate samples from the periplasmic space sample. No activity was detected in samples from pBR322. The sensitivity minimum of the assay as assessed from the standard curve with authentic GH was 10 ng ml^{-1}. The high immune reactivity in the periplasmic space is probably due to lysis of some cells during spheroplast formation.

The construction of a hybrid gene between bacterial and mammalian DNA sequences, capable of directing synthesis of rat growth hormone (RGH) sequences in bacteria, is shown in Fig. 1. The bacterial sequences for this gene were excised from the multicopy plasmid pBR322 (ref. 9). They contain a portion of the gene (*amp*^{r}) coding for the enzyme β-lactamase which is secreted into the periplasmic space and is responsible for bacterial resistance to the antibiotic ampicillin[9]. The mammalian sequences were provided by cloned complementary DNA containing the entire coding region for the precursor form of rat pituitary GH[2]. Functional fusion of the two donor DNAs was facilitated by knowledge of their primary structures[2,10]. Both DNAs possess a unique cleavage site for the restriction endonuclease *Pst*I at which they can be joined to maintain the correct reading frame (Fig. 2). In the *amp*^{r} gene the *Pst*I site is situated approximately two-thirds towards the end. In RGH cDNA the corresponding site occurs a few base pairs distal to the

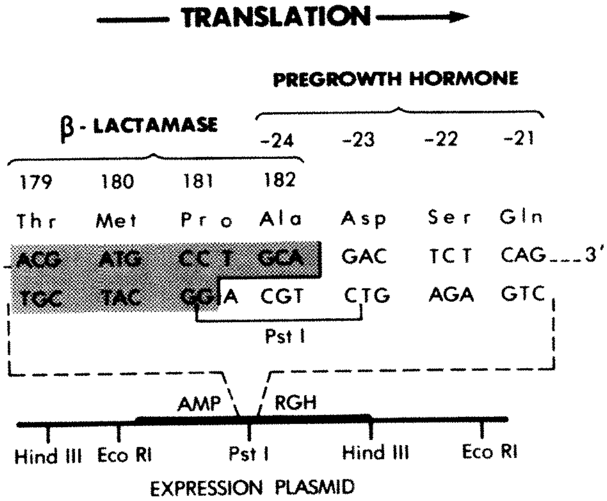

Fig. 2 Postulated nucleotide sequence around the *Pst*I site in the hybrid gene. The *Pst*I site is at the fusion between the *amp*^{r} gene sequences[10] and the cloned RGH cDNA[2]. Positive numbers refer to amino acid residues of pre-β-lactamase and negative numbers to those of the RGH pre-sequence.

initiator codon for the pre-peptide. The resultant new gene should code for a chimaeric protein of 395 amino acids (molecular weight $\sim$44,000) containing the N-terminal 181 amino acids of the β-lactamase precursor covalently linked to 214 residues of rat pre-GH. As this fusion protein carries the signal peptide of β-lactamase, it seems possible that it might be transported to the periplasmic space. Such an extracellular location might minimise possible protease attack and facilitate isolation.

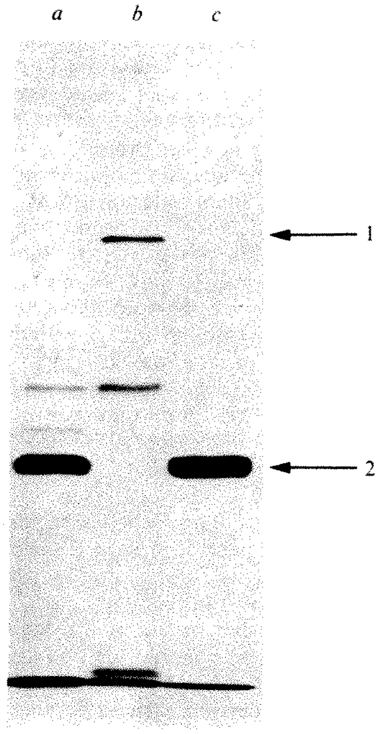

Fig. 3 Proteins synthesised from plasmids pEx-RGH and pBR322. The minicell-producing *E. coli* strain P678-54 (ref. 13) was transformed with pEx-RGH or pBR322 by standard methods[2]. A culture of each (200 ml) was grown to the late log or stationary phase of growth ($A_{650} = 0.7$) in L broth containing 0.5% glucose. Minicells were purified by repeated zone sedimentation through 5–25% sucrose gradients as described elsewhere[12] and resuspended in 2 ml of leucine- and methionine-free M-9 salts medium. After 30 min preincubation at 37 °C, 250 μCi of ^{35}S-methionine and 50 μCi of ^{3}H-leucine were added to both cell preparations, which were then vigorously shaken for 30 min at 37 °C. After a 30-min chase period with 400 μg of nonradioactive methionine and leucine, the minicells were pelleted by centrifugation at 8,000*g* for 2 min and half the cells of each preparation were lysed in 200 μl of SDS sample buffer[14]. The second half of each minicell preparation was used to isolate the proteins secreted into the periplasmic space as described elsewhere[15]. Labelled proteins isolated from equivalent amounts of minicells were electrophoresed on a 10% polyacrylamide–SDS gel[14] and identified by autoradiography of the dried gels. Lanes *a* and *b* represent the total proteins synthesised by pBR322 and pEx-RGH, respectively. Lane *c* reflects the proteins secreted into the periplasmic space of minicells carrying pBR322. Arrow 1 refers to the location of the fusion protein and arrow 2 indicates β-lactamase.

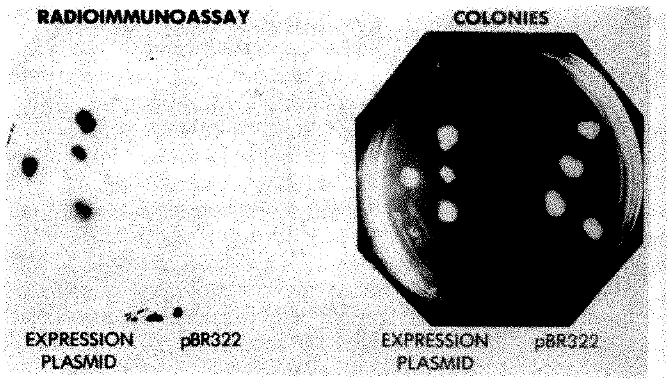

Fig. 4 Immunological detection of RGH sequences in bacteria. Bacterial colonies containing the constructed expression vector pEx-RGH were assayed for the presence of RGH sequences using a modification[16] of the solid-phase immunological screening method Broome and Gilbert[17]. The described wash buffer was replaced with phosphate-buffered saline (0.1 M NaCl, 0.025 M potassium phosphate, *p*H 7.4) containing gelatin (10 mg ml^{-1}) and NP40 (0.1%). Bacterial colonies of *E. coli* strain P 678-54 (ref. 13) carrying pEx-RGH or pBR322 were grown on L-broth agar[18] in Petri dishes. A photograph of such a plate is shown on the right. After treatment (20 min) with chloroform vapour the colonies were exposed to polyvinyl strips coated with antiserum to RGH. Strips were then soaked in the modified wash buffer (see above) to minimise binding of IgG in the subsequent reaction. The strips were then incubated overnight at 4 °C with affinity column-purified ^{125}I-anti-RGH IgG (10^{6} c.p.m., specific activity $\sim$5.3 μCi per μg), washed, and exposed to X-ray film. An autoradiogram is shown on the left.

To enable selection of plasmids carrying the correct hybrid gene, the cloned GH cDNA (originally isolated in plasmid pBR322) was first transferred from the *Hin*dIII site of pBR322 into the homologous site of pMB9, a closely related plasmid which lacks the amp^r gene. In the resultant recombinant plasmid (pMB9-RGH) the gene for tetracycline resistance is expressed at reduced levels[11]. One such plasmid which contains the GH cDNA inserted in the orientation shown in Fig. 1 was used as the recipient of amp^r gene sequences. Replacement of the unique *Pst*I–*Bam*HI fragment in pMB9-RGH with that from pBR322 should place the coding sequences for rat GH under control of the amp^r gene and will at the same time restore a fully functional tet^r gene (see Fig. 1). Thus, this fragment replacement enabled selection of bacterial colonies carrying the expression vector 'pEx-RGH' by their ability to grow on high levels of tetracycline.

Gene expression

Purified minicells[12] were used for the study of proteins encoded by the expression vector pEx-RGH. Proteins were analysed by SDS–polyacrylamide gel electrophoresis and compared with those encoded by pBR322 (Fig. 3) and pMB9 (data not shown). As anticipated, the two polypeptides pre-β-lactamase and β-lactamase which are synthesised in cells carrying pBR322 are not made from pEx-RGH. The expression vector codes uniquely for a polypeptide (Fig. 3, lane *b*, arrow 1) with an apparent molecular weight (46,000) within error limits of that expected for the hybrid protein. A comparison of band intensities (corrected for cell number) from several experiments similar to that described in Fig. 3 suggests that the 46,000 MW protein is produced in substantial quantities from pEx-RGH, although in lower amounts (about one-fifth) than β-lactamase from pBR322. When proteins released after the formation of spheroplasts in high osmotic pressure medium[15] were analysed by gel electrophoresis, a large portion of β-lactamase (Fig. 3) but not of the pEx-RGH specific product was detected. The same situation applied when material from media of whole cells was examined (data not shown). Thus, contrary to the case with β-lactamase, the 46,000 MW protein was present only in very small amounts in the periplasmic space if at all.

Immunological detection of growth hormone synthesised by bacteria

Escherichia coli colonies containing pEx-RGH were screened for their content of RGH using a modification[16] of a solid-phase munological method[17] (Fig. 4). Briefly, colonies were lysed *in tu* with $CHCl_3$ and any RGH sequences present in the cells ere bound to a polyvinyl disk coated with IgG purified from tiserum to RGH raised in monkeys (see ref. 16 for details on eparation of antiserum). Bound antigen was then specifically belled with highly purified ^{125}I-anti-RGH IgG (see legend to ig. 5) and colonies producing RGH were visualised by toradiography. As shown in Fig. 4, colonies containing the pression plasmid were labelled with the ^{125}I-anti-RGH-IgG obe, whereas those containing pBR322 failed to do so.

Several controls demonstrated the immunological specificity f the assay for RGH and strengthened the conclusion that the ones are producing pituitary GH sequences (Fig. 5). First, lonies of the K12 strain of *E. coli* devoid of plasmid were not belled with the ^{125}I-anti-RGH-IgG probe. Second, labelling of sates of colonies containing pEx-RGH was prevented when a rge excess (250-fold) of nonradioactive GH was incubated ith radioactive probe. Third, labelling was not observed when ormal monkey serum or calf serum (data not shown) were used place of anti-RGH antiserum. Finally, ^{125}I-labelled normal onkey serum failed to label any colony from the strains escribed above (not shown).

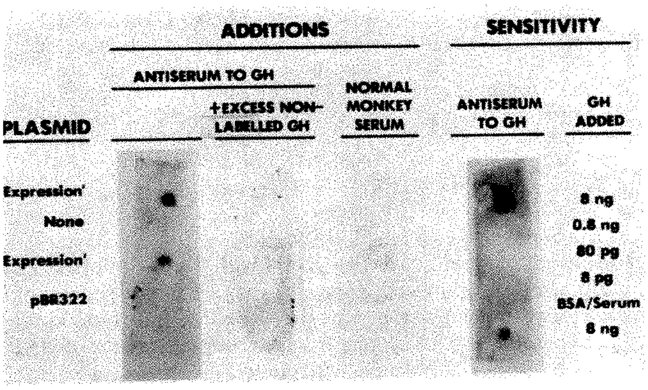

Fig. 5 Immunological detection of GH sequences synthesised by bacteria: specificity of the assay. In 'antiserum to GH', the colonies were treated as described in the legend to Fig. 4. 'None' refers to colonies of *E. coli* devoid of plasmid. In other experiments ('+ excess non-labelled GH'), a large excess (250-fold, 5 μg) of highly purified RGH was preincubated with ^{125}I-anti-RGH IgG. The autoradiograph shown in the lane 'normal monkey serum' results from using polyvinyl strips coated with normal monkey serum (in place of RGH antiserum). A slight reaction occurred with cells containing pEx-RGH, as antigen (RGH) sticks to plastic even in the absence of anti-RGH IgG. The sensitivity of the assay was measured by applying varying amounts of pure RGH (8 ng, 0.8 ng, 80 pg, 8 pg and 8 ng) to an agar plate and screening as described above.

The sensitivity of the screening procedure is indicated in Fig. 5. Labelling was observed with 8 ng, but not with 0.8 ng, of uthentic RGH added to the plate. Thus, each colony seems to ontain several nanograms of GH sequences, although further xperiments are needed to obtain better quantitation.

As indicated in Table 1, immune reactivity was also detected by direct radioimmunoassay in extracts of spheroplasts and in material from the periplasmic space. No such activity was observed in similar isolates from bacteria containing pBR322.

Discussion

In the present studies, bacteria have been genetically programmed to synthesise mammalian pituitary growth hormone sequences. Further analysis is required to assess the fidelity of such expression, the stability of the protein products, and their biological activity. The absolute quantity of GH synthesised by the bacteria cannot be determined accurately from the current studies (an estimate of 24,000 molecules per cell is obtained from the solid-phase immunoassay). However, judging from the prominence of the band in Fig. 3, it seems that sequences of RGH can be a major plasmid product. The quantity of GH present is less than that of β-lactamase; however, the study shown in Fig. 3 may result in an underestimate of the amount of GH synthesised, because in more recent studies (not shown), material of lower molecular weight (in addition to the larger molecular weight material) is specifically immunoprecipitated by antiserum to RGH but not by antiserum to bovine serum albumin. This material could consist of degradation products or the products of premature termination.

The *amp*^{r} gene was used for expressing RGH coding sequences with the hope that the mammalian hormone would be secreted by the bacteria. Such secretion might facilitate the isolation of the protein product and also protect the protein from degradation by proteases. The absence of GH sequences in the periplasmic space is surprising in view of the fact that Villa-Komaroff *et al.*[20] have recently constructed a gene suitable for the expression of rat insulin through linkage with the *amp*^{r} gene and found that the protein product was secreted[20]. It is possible that the hydrophobic region corresponding to the pre-part of RGH in the middle of the hybrid protein prevents the kind of conformation required for transport through membranes. Similarly, the overall structure of the fusion polypeptide might prevent correct processing of the precursor sequence. More detailed studies should help determine which factors, other than pre-sequence of the signal peptide protein of the hormone, are necessary for secretion.

Our findings and those of Villa-Komaroff *et al.*[20] indicate that coding sequences from higher organisms can be expressed in bacteria. Thus, it should be possible to produce biologically important peptides with the use of recombinant DNA techniques and naturally occurring structural gene sequences. Finally, the capability of expression of genes that are designed specifically or synthesised[21] should make possible the production of new hormone analogues for examining the structural requirements for agonist or antagonist activity or for therapy.

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letters

Discovery and optical identification of 2S0921 – 630

THE discovery of a faint X-ray source with the X-ray detectors on SAS 3 at a location with galactic coordinates $l^{\text{II}} \sim 282°$, $b^{\text{II}} \sim -9°$ is reported here. A subsequent optical search has revealed a likely optical counterpart in the form of a 17th magnitude star with He II 4686 and Hβ in emission. It is, therefore, likely to be a low-luminosity galactic X-ray source.

From 10 to 14 February 1978, the rotation modulation collimator X-ray detectors[1] on SAS 3 were used to search for X-ray emission from a region centred at $\alpha \sim 135°$, $\delta \sim -67°$. Data were accumulated from a total exposure of 130,000 s. The correlation maps show a previously unreported X-ray source, which we designate 2S0921 – 630 at α = 09 h 21 min 25.4 s, $\delta = -63°04'27''$ (1950 Equinox) with an error circle of radius 30″ (90% confidence). Figure 1 is an enlarged reproduction from the ESO (B) survey print with the error circle superposed. The average flux density of the source during the observation was 2.3 ± 0.3 μJy at 5.2 keV (1 μJy $= 0.242 \times 10^{-11}$ erg cm^{-2} s^{-1} keV^{-1}).

Optical observations of stars in the above error circle were made with the 3.6 m telescope of the European Southern Observatory (ESO). To search for a candidate counterpart with possible strong Hα emission, one of us (S.L.) took a direct prime-focus plate, through a 30 Å wide Hα filter, of the region of the sky centred on the error box. Star No. 5 in Fig. 1 is remarkably bright on this plate. We estimate from the ESO survey print that its photographic magnitude is approximately 17.

Spectrograms (60Å mm^{-1}) were taken (by J.v.P.) of this star with the Carnegie image tube attached to the Boller and Chivens Cassegrain spectrograph. The plates were taken on 13 March 1978, 03.33 UT and 04.07 UT and have exposure times of 40 and 20 min, respectively. IIa–O (baked) plates were used and the spectra have a widening of 0.2 mm. They cover the wavelength range from ~3,700 to ~5,000 Å. Density tracings of the spectra were made with the Faul–Coradi microphotometer of the Observatory of Utrecht. The only features clearly visible in the spectrum are a strong He II 4686 and a weak Hβ line, both in emission. Strong He II emission lines are generally present in the spectra of the counterparts of X-ray sources (see refs 2 and 3) but not usually in those of normal stars; on the basis of the above characteristics we consider star No. 5 a likely candidate for the optical counterpart of 2S0921 – 630.

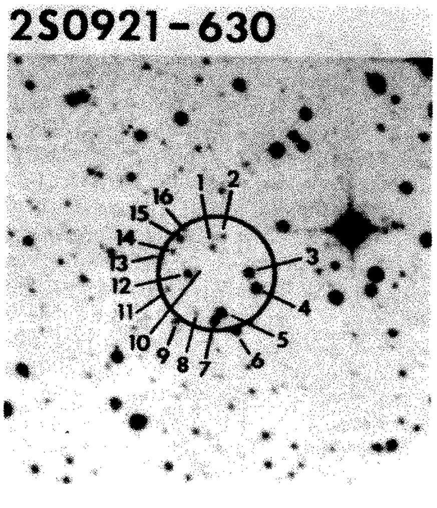

Fig. 1 Error box of 2S0921 – 630 superposed on the ESO survey print. Star No. 5 is the proposed optical counterpart.

The optical spectrum of this object is similar to the spectra of the optical counterparts of Sco X-1 and the similar galactic X-ray sources that have been discussed by McClintock *et al.*[2]. It is interesting, however, that the ratio of X-ray to optical luminosity for 2S0921 – 630 is ~10 which is much smaller than the ratios for other members of this class of counterparts which are typically of the order of 1,000. The X-ray luminosity of the sources is $\sim 6 \times 10^{35}$ $(D/10 \text{ kpc})^2$ erg s^{-1}. It may belong to the class of low luminosity galactic X-ray binaries.

We thank the staff at MIT Center for Space Research for help with this observation. Permission to use the Faul–Coradi microphotometer is gratefully acknowledged.

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Simultaneous radio and X-ray observations of MXB1837 + 05 (Ser X – 1)

DURING a continuing program of radio observations of X-ray sources carried out with the NRAO very large array (VLA), we have made high sensitivity searches for radio emission from MXB1837 + 05 = Ser X – 1 (ref. 1), particularly during the SAS 3 MIT world-wide X-ray burster watch in June 1977[2]. Observations of MXB1837 + 05 were carried out on 26 April, 12 and

13 June 1977, with six 25-m antennas of the VLA operated at 4,884 MHz with system temperatures of $\sim$60 K and bandwidths of 50 MHz. Upper limits of 1 mJy were obtained for the time-averaged radio emission from Ser X$-$1 during the three periods of observation, and no short time scale radio emission was detected during or after the times X-ray bursts were observed on 12 and 13 June 1977.

The times of observations with the VLA were from 06.01 UT to 13.50 UT on 26 April 1977, from 06.15 to 13.18 UT on 12 June and from 07.04 UT to 13.14 UT on 13 June 1977. During these periods calibration sources were observed once every half hour. The VLA data were accumulated in 30-s integration intervals, and after calibration, were used to make radio maps of about 4 arc min square regions with 2 arc s resolution. The centre of each map was taken to be the position of MXB1837+05 ($\alpha(1950) = 18\text{h}37\text{min}29.8\text{s}$, $\delta(1950) = +04°59'23''$) which was known to about 20″ (ref. 3). Maps were made from various subsets of each 7-h period allowing upper limits to be set for variable radio emission on time scales from 30 s up to $\sim$7 h. On each of the three days the upper limits to the time-averaged radio emission anywhere in the fields of the map were $\sim$1 mJy. The times of major interest were the periods during and after X-ray bursts observed by SAS 3 on 12 and 13 June. The upper limits placed on radio emission during and after the bursts are a factor of 8 lower than the limits established by Johnson *et al.*[4] for X-ray bursters including MXB1837+05 and a factor of 100 lower than the limits found by Thomas *et al.* for MXB1636$-$53.

The X-ray observations were made in the 1.3–19-keV energy range with the SAS 3 horizontal tubes and xenon tube system[6,7]. Two X-ray bursts were observed during VLA radio coverage on 12 June at 11.22.20 and 13 June at 9.29.27 UT. The X-ray bursts lasted $\sim$17 s with average X-ray fluxes of 0.2–0.4 mJy. Maps with integration times of 30s, 10 min, 2 h, and 7 h were made from the radio data, with upper limits for any sources in the field of 15, 4, 1.5 and 1.0 mJy, respectively, with the shorter time periods taken coincident with or after the X-ray bursts. Exact simultaneity was achieved with the 12 June burst at 11.22.20 UT, but the 13 June burst at 9.29.27 UT occurred during a 5-min gap when a calibrator was being observed. With the limits mentioned above for the four time scales of mapping, no radio emission was observed during or after the 12 June burst and, no radio emission was observed before or after the 13 June burst.

In conclusion, even very high sensitivity radio observations cannot detect bursting X-ray sources. A clearer picture of these objects is needed, however, before the radio observations can effectively be discussed on a theoretical basis.

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Interplanetary dust: are there two independent populations?

A RE-EXAMINATION of the information on the size distribution and physical properties of interplanetary dust grains as inferred from space measurements, particularly lunar microcraters, leads to the novel interpretation outlined here in terms of two independent populations. This hypothesis still suffers from uncertainties in existing data but should stimulate new experimental and theoretical work.

New data[1–5] on lunar microcraters together with the review of Fechtig *et al.*[6] have firmly established the following features of the cumulative size distribution function $F_c(D_c)$ usually represented by a D_c^{-q} power law: (1) a power index $q = 3.5$ for craters >100 μm; (2) a progressive decrease of q with decreasing diameter, starting at $D_c \simeq 100$ μm, and ending with a marked inflection point around $D_c \simeq 5$ μm; (3) a new high power index, $q = 3.1$, for microcraters smaller than $\sim$5 μm down to 0.1 μm.

The second point has already been interpreted as evidence for a bimodal population[7]. The third point was recognised only recently[1,4] when it was realised that accretionary particles and ejecta resulting from larger impacts are extremely efficient in masking the smallest microcraters. Note that microcraters with diameters as small as 0.05 μm have been observed[8]. The ratio of crater depth to crater diameter, P/D_c, offers a direct means of probing the density of impacting micrometeorites: although they are still limited to simple experimental conditions, calibrations have shown that P/D_c assumes well defined values characteristic of the density of the projectile[9,10] (Fig. 1). However, due to conflicting results[11], no clear conclusions have been reached on this matter. For instance, Brownlee *et al.*[7,12] and Mandeville[13] found a mean density of approximately 3 g cm^{-3} whereas Smith *et al.*[10], Durrani *et al.*[14], and more recently Nagel *et al.*[2] detected up to three densities: 1, 3 and 8 g cm^{-3}. On the basis of a global study of the published data regrouping 256 microcraters, we propose a clarification of this contradiction as the result apparently depends on the crater sizes (Fig. 1). In particular, by separating them into different size ranges, we found that the apparent contradictory data of Brownlee *et al.*[7,12] and Smith *et al.*[10] were in satisfactory agreement.

From our analysis (Fig. 1), the following conclusions can be put forward. First, low density grains ($\leqslant 1$ g cm^{-3}) are not detected; second, two families coexist, the large grains having, exclusively, densities typical of silicates and the small ones densities typical of metals with a minor component of the first family. In the intermediate range ($1 < D_c < 10$ μm) the two families are mixed. It is precisely in this range that the cumulative distribution function F_c presents an anomalous behaviour (inflection point) which was an indication for a bimodal population[7]. The *in situ* measurements performed by the Pioneer 8 and 9 and Helios 1 space probes have also conspicuously shown two families of dust grains; the first consists of large grains travelling on nearly circular orbits, whereas the second is composed of small grains, commonly named β-meteoroids[17], leaving the Solar System in hyperbolic orbits[15,16]. Doppler shifts measurements in the Zodiacal Light Spectrum[18] have confirmed the existence of both types of orbits. The separation between these two families again takes place for particle sizes in the μm range. Collection experiments of extraterrestrial particles performed by Brownlee *et al.*[19] during stratospheric flights have revealed two distinct kinds of grains: (1) large compact aggregates of submicronic grains having chondritic abundances and a mean density of 2 g cm^{-3}; (2) smaller, homogeneous, nearly spherical 'iron–sulphur–nickel' (FSN) grains.

The cumulative flux $F(s)$ of interplanetary dust versus grain diameter may be obtained from $F_c(D_c)$, provided the time of exposition and the calibrations of the ratio D_c/s are known. From the work of Morrison and Zinner[1] on sample 12054,54, we used a time of $1.75\times10^5 \pm 5\times10^4$ yr and a constant value $D_c/s = 2$ from the calibration of Mandeville[20] and Bloch *et al.*[21] to obtain the result presented in Fig. 2.

All the above results are therefore coherent with a two-component model of interplanetary dust: Population 1 consists principally of large grains ($s > 2$ μm) of density typical of silicates or chondritic materials (2–3 g cm^{-3}) in nearly circular orbits while Population 2 consists of small grains ($s < 2$ μm) with typically metallic densities (8 g cm^{-3}) in hyperbolic orbits. The dip observed in $F(s)$ (Fig. 2) is readily interpreted as the gradual disappearance of Population 2 which is more evident on the differential size distribution sketched in Fig. 3.

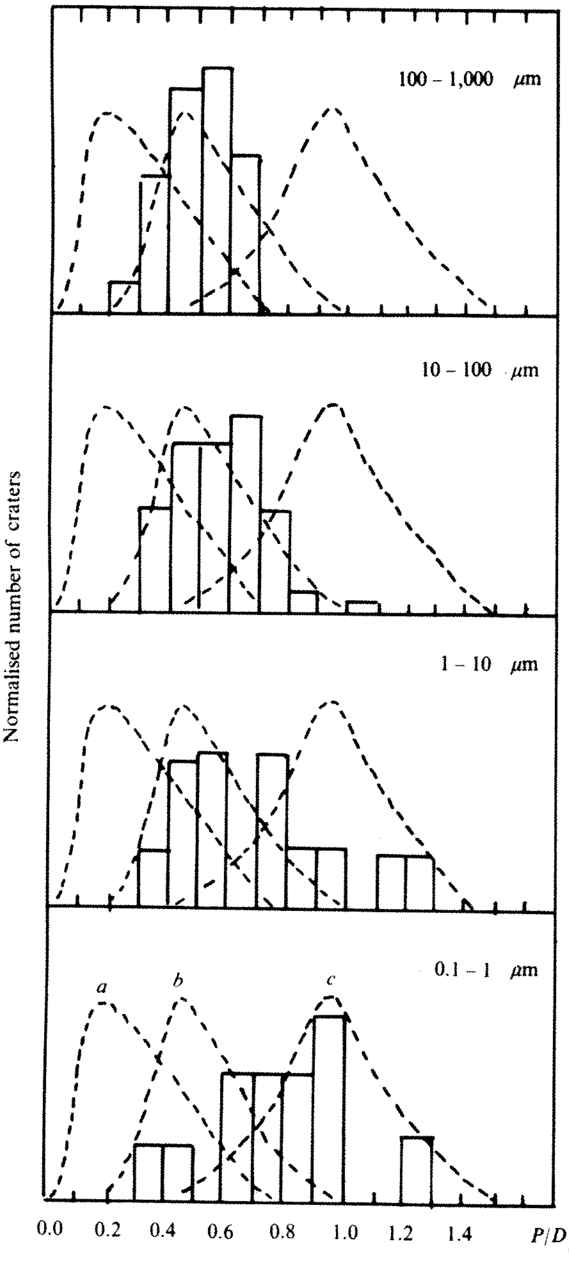

Fig. 1 Normalised histograms of the values of the ratio P/D_c for 256 craters separated in four diameter ranges as indicated. Superimposed (dashed lines) are three simulation curves corresponding to three different densities of impacting projectile. *a*, 1 g cm^{-3} (polystyrene); *b*, 2.7 g cm^{-3} (aluminium); *c*, 7.8 g cm^{-3} (iron).

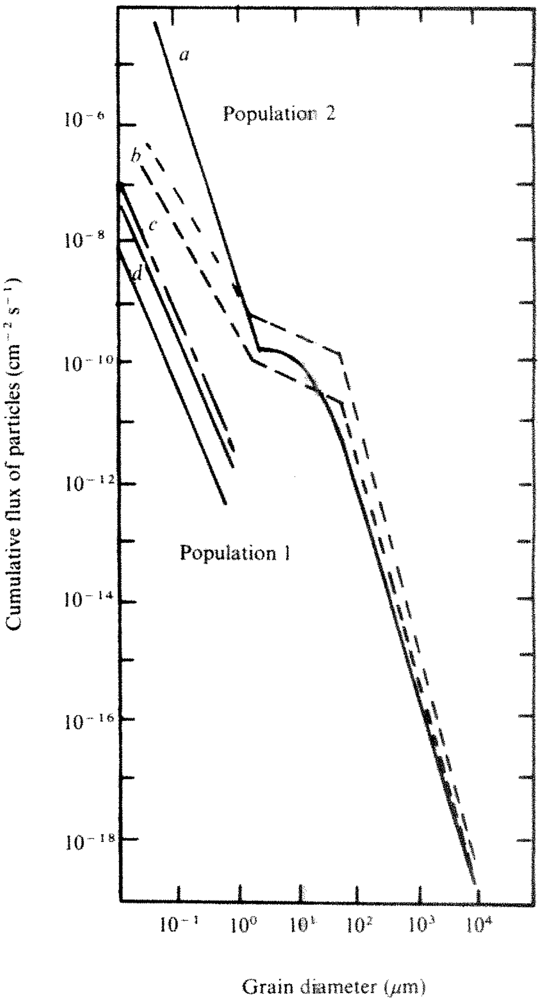

Fig. 2 Cumulative flux plotted against diameter of the particles arriving on the moon: *a*, present result; *b*, results of Fechtig *et al.*[6]; *c*, result derived from the conservation of mass flux; *d*, result from the collisional model[26].

There have been attempts to explain the origin of β-meteoroids[17,22] (which we identify with Population 2) based on the collisional fragmentation of larger grains (Population 1) within 1 AU and repulsion of the submicronic fragments under the radiation pressure force. The different physical nature of the two populations deduced from lunar microcraters casts some doubt on this interpretation which may be further rejected on the basis of the conservation of mass flux, an argument which bypasses the difficulties inherent in collisional models of grains (lack of direct calibrations).

Following the model of Zook and Berg[17], Population 1 grains are supposed to spiral slowly towards the Sun under the Poynting–Robertson effect: they may be considered to travel in nearly circular orbits and to collide because of different inclinations. If $f(s) = -\mathrm{d}F(s)/\mathrm{d}s$ denotes the differential flux of grains measured on the Moon, the spatial density is $f(s)\,\mathrm{d}s/\langle V\rangle$ where $\langle V\rangle$ is the mean velocity of the grains. The inward drift yields a radial velocity[23] $V_{PR}(s)$ leading to a radial flux of grains:

$$V_{PR}(s) f(s)\,\mathrm{d}s/\langle V\rangle$$

The total mass influx at 1 AU is therefore

$$\Phi_1 = \frac{\pi}{6}\frac{\rho}{V}\int_{s_{1,\min}}^{s_{1,\infty}} s^3 V_{PR}(s) f(s)\,\mathrm{d}s$$

where ρ is the density of the grains, $s_{1,\min} \simeq 2$ μm is the minimum diameter, and $s_{1,\infty}$ is the maximum diameter of Population 1 grains.

The situation is entirely different for Population 2 grains for which the radial velocity is of the order of the orbital hyperbolic velocity. The total mass outflux at 1 AU is simply

$$\Phi_2 = \frac{\pi}{6}\rho \int_{s_{2,\min}}^{s_{2,\infty}} s^3 f(s)\, \mathrm{d}(s)$$

where $s_{2,\min} \simeq 0.01$ μm is taken as the minimum size of Population 2 grains and $s_{2,\infty} \simeq 2$ μm as the maximum size. The final value of Φ_2 is not sensitive to this choice of parameters. The collisional hypothesis requires

$$\Phi_1 \geqslant \Phi_2$$

while we found

$$\Phi_1 \simeq 5\times10^{-23}\ \mathrm{g\ cm^{-2}\ s^{-1}} \quad \text{and} \quad \Phi_2 \simeq 5\times10^{-20}\ \mathrm{g\ cm^{-2}\ s^{-1}}$$

The difference between the two fluxes is so large that our present conclusion will not be affected by any possible variations of the parameters involved in the calculation (grain density, orbital velocities) not by uncertainties in the exposure time which do not affect the ratio Φ_1/Φ_2. Using the above value of Φ_1, we can give an upper limit for the flux of fragments resulting from collisional destruction under the constraint $\Phi_2 = \Phi_1$ assuming further that (1) all grains of Population 1 are destroyed and all fragments smaller than 2 μm are ejected on hyperbolic orbits; (2) the distribution of fragments follows a power law $s^{-\eta}$ where $\eta = 3.48$ (refs 24, 25). The result is shown in Fig. 2 and is three orders of magnitude smaller than the observed flux. We constructed an elaborated collisional model[26] using the basic procedure of Dohnanyi[22] but incorporating as many real data as possible[19,25]. The model fluxes of fragments always lie slightly under the upper limit deduced from the conservation of mass flux. If one accepts the present results from lunar samples and space experiments, we arrive at the conclusion that the two populations are entirely independent on several grounds: size, density and dynamics.

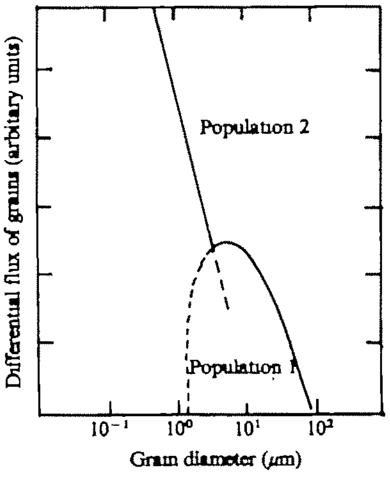

Fig. 3 Differential flux of particles as a function of particle diameter (arbitrary units)

Population 1 has probably its origin in dust grains ejected by short period comets. The sizes and size distribution law are in surprisingly good agreement with those derived for Comet d'Arrest[27]. The bulk of Population 1 grains probably disappear within 1 AU as the value of β (ratio of the radiation pressure to the gravitational forces) for silicates[28] and the 'initial' orbital conditions at 1 AU (nearly circular orbits) ensure that they will not escape the inner region of the Solar System whatever processes (collisions, sublimation) they undergo. We may therefore consider that Φ_1 corresponds to the mass loss rate of Population 1. Integrating Φ_1 taking into account the distribution of inclination[22], we found a mass loss rate of 2.8×10^4 g s^{-1}. To maintain Population 1 in steady state, approximately 70 short-period comets similar to Comet d'Arrest is required on the basis of an average production rate of 400 g s^{-1}. Note that this satisfactory number is obtained assuming a grain albedo of 0.5, a more reasonable value than the 0.001 considered by Sekanina and Schuster[27]. Population 2 presents far more difficult problems. Either the grains are formed in the solar atmosphere and subsequently expelled by radiation pressure[29] (although this presents difficulties[30]) or they are still contained in larger bodies when they penetrate inside 1 AU. Long-period comets could be a possible source as they are known to be very dust productive. However, the existence of only prograde hyperbolic orbits[18] seems to rule out this solution to a large extent.

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Is the Sun almost-intransitive?

THERE is now ample instrumental, historical and proxy evidence for palaeoclimatic variability over time scales ranging from 10 to 10^9 yr (ref. 1). Although a great deal of research is still needed to establish more precisely the nature and finer spatial and temporal resolution of these changes, the main theoretical problem is to understand the mechanisms responsible for this variability over such a wide range of time scales. We suggest here that if the Sun were almost-intransitive, it could explain some of this climatic variability. Many hypotheses have been advanced by researchers in various disciplines based on physical processes, both terrestrial and extra-terrestrial[1–8], and although there is no general consensus as to the causes of variability over various time scales, the common view among climatologists is that the very long time-scale fluctuations with periods of ~10^8 yr can be explained by causes of terrestrial origin, such as the changes in oceanic and continental geometries[3,10]. The intermediate time-scale fluctuations with periods of $\sim 10^4$–10^5 yr are thought to have been caused by variations in Earth's orbital elements[9]. The relatively short time-scale fluctuations with periods of $\sim 10^2$–10^4 yr are, on the other hand, hard to explain, because of the clear lack of a physical process with the appropriate time scale. Solar variability is, however, accepted as a serious contender[3,10]. All these hypotheses, whether terrestrial or extra-terrestrial, have in common the assumption that the changes in climate are deterministic, that is, they are a causal response of the climatic system to a change in some environmental (internal or external) parameter, even though the actual mechanism of change is not usually understood.

A radically different hypothesis by Lorenz, suggests that the climatic variability over some time scales may turn out to be non-deterministic[14–16]. This possibility arises, essentially, from the fact that the climate can be defined as a set of long-term statistics of the time-dependent solution of a set of appropriate non-linear differential equations. On the basis of ergodic theory, both transitive systems (ones in which long-term statistics of some time-dependent solution is independent of the choice of boundary conditions) and intransitive systems (ones in which the solution is dependent on the choice of the boundary conditions) can exist[16]. Evidence for intransitive systems also exists both experimentally[17] and numerically[14]. In addition to the above two modes of behaviour for a system, Lorenz has pointed out the possibility that a system of non-linear differential equations may have solutions which exhibit different statistical properties within different segments of a long time span, with a completely constant environment. Such systems he has termed almost-intransitive[14–16]. As an example of these systems, he has constructed numerical models (which to some extent resemble the numerical models of the Earth's atmosphere, or for that matter any similar non-linear system of differential equations), which behave in an almost-intransitive fashion[16].

There is also some evidence to suggest that the climate may have behaved in an almost-intransitive fashion over time scales with periods of $\sim 10^4$ yr (ref. 18). It may, therefore, seem likely that the possible almost-intransitivity of the climatic system could account for climatic variability over such time scales, without the need to invoke solar variability. This, however, does not seem to be the case, as there are strong indications of solar variability over these time scales, both historical[12] and proxy. The latter evidence is based on the measurements of radiocarbon(^{14}C) fluctuations in tree rings which are thought to have been caused by the solar modulation of galactic cosmic rays[11]. There is also some apparent correlation between the radiocarbon variations and the historical climatic record[10,11,19,20]. Solar variability over such time scales cannot, therefore, be ruled out in favour of almost-intransitivity of the climatic system.

There is, however, some difficulty in accounting for the solar variability over these time scales. This becomes apparent when one takes a closer look at the commonly accepted solar model (with its own difficulties[21]), and the time scales characteristic of the physical processes that are thought to be operative in the Sun. Such examination reveals a spectrum of time scales dominated on one end with very long time scales of $\sim 10^7$–10^{13} yr, and on the other with very short time scales of $\sim 10^{-4}$–10^{-1} yr (ref. 13). Therefore within the context of the presently accepted solar model, the time scales of variability for which there is some evidence and are thought to be important climatologically, namely those with periods of $\sim 10^2$–10^4 yr, are hard to explain. It therefore seems to be necessary to reconcile the evidence for solar variability over such periods, the lack of physical processes with appropriate time scales operative in the Sun and the possibility of the existence of systems which behave almost-intransitively.

We suggest that this could be achieved if the Sun itself (its coupled 'convective zone-atmosphere') were almost-intransitive. Some of the arguments that support the plausibility of this hypothesis and some of its consequences are given below.

The complete, unapproximated fluid dynamical equations representing the convective zone-atmosphere of the Sun are highly non-linear and are very similar to those representing the Earth's atmosphere. Lorenz's arguments regarding the plausibility of the almost-intransitivity of the climatic system could therefore equally apply to the Sun, or to any physical system where similar highly non-linear differential equations are operative.

Almost-intransitivity of the Sun could account for the evidence of solar variability in radiocarbon data and in the historical record, and also the climatic variability over these time scales. Almost-intransitivity of the climatic system on the other hand, although possible, cannot account for the independent evidence for solar variability over such time scales.

There is also the possibility, on the basis of dishpan experiments[17] with their limited similarity to the climatic system, that the climatic system may be intransitive[16]. If so, it is hard to switch from one climatic regime to another without the presence of an external forcing mechanism[16]. Almost-intransitivity of the Sun could act as such an external forcing agency.

It seems plausible therefore that the Sun could be almost-intransitive over some time scales. It should, however, be added that the climatic system is highly non-linear and complicated, and it is possible that even if the Sun is almost-intransitive, this need not be the sole cause of climatic variability over such time scales. Nevertheless, if the Sun turns out to be almost-intransitive it could have astronomical as well as climatological significance.

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Waves in narrow channels: faster capillary waves

DURING experiments on the propagation of water waves in channels with parallel sides made of Perspex, it was found that the phase velocities of small-amplitude waves with wavelengths in the range 40–100 mm were consistently higher than predicted by the standard hydrodynamic theory[1,2]. The original observations of this anomaly were made in a channel 100 mm wide filled to a depth of 50 mm. For these none too small dimensions, the standard theory might be expected to provide a close approximation to the wave velocity, but velocities $\sim 0.5\%$ too high were observed. Highly surface-clean water and clean apparatus was used in all the experiments, and with these

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precautions the measurements proved to be wholly reproducible, prompting a closer examination, which is described here, of the wave motion to detect the cause of the anomaly. It was seen that the lines of contact between the water surface and the sides of the channel did not in fact rise and fall in step with the movement of the surface at the centre of the channel, instead remaining fixed in their original locations with the water at rest.

The observed immobility of the contact lines exemplifies the phenomenon commonly called contact-angle hysteresis. A larger contact angle is apparent when a liquid is advancing over a solid surface than when it is receding; and correspondingly, for a static contact line, there is an intermediate range over which the angle can vary to counterbalance forces tending to dislodge the line. This hysteresis, which often spans a sizable fraction of the mean contact angle, may be observed easily with almost every practical (non-reacting) combination of liquid and solid, and it is usually due to either microscopic roughness or chemical inhomogeneity of the solid surface.

With regard to experiments such as ours, therefore, it is reasonable to conclude that contact lines will remain fixed, provided the wave amplitude does not exceed a certain threshold. The vibrating surface of the water will then curve like a membrane tied at its edges, and the capillary pressures associated with the additional, transverse curvature will evidently stiffen the surface, so increasing the velocity of wave propagation. Further experiments were made using much narrower channels, for which the stiffening effect was expected to be correspondingly greater, and phase velocities were recorded that were remarkably higher than those of the more familiar, long-crested waves in the same range of wavelengths. For example, in a channel 4 mm wide filled to a depth of 25 mm, the phase velocity of waves 100 mm long was found to be about 2.5 times the predicted value for waves of the same length free from edge constraints.

A linearised theory for the new class of wave motions has been developed, focussing on the case of an exactly brimful channel. An advantage of this case as a theoretical model is that the free surface is flat when at rest, so that evaluation of the capillary pressures on the perturbed surface is comparatively simple. It also has considerable practical advantages, in particular that the contact lines can be located securely along sharp edges of the channel and that the required flatness of the free surface can be judged accurately by viewing the mirrored images of distant objects; accordingly, it was made the subject of the final, most exacting series of experiments devoted to these waves. Other assumptions of the theory apparently compatible with the experimental conditions are that the liquid is incompressible and inviscid, so that its motion started from rest is irrotational, and that the liquid has a constant surface tension.

The theory is more complex than the corresponding theory for waves free from edge constraints; and even in the simplest case where the channel has a rectangular cross-section, explicit solutions are not available. As usual in water-wave theory, the hydrodynamic loading of the perturbed free surface can be represented abstractly by a Neumann boundary-value problem, but the edge conditions imposed on functions representing the displacement of the free surface imply that no finite combination of the eigenvectors of this constituent problem can solve the complete problem including the effects of surface tension. A variational formulation leads, however, to a well-defined expression, in the form of a Rayleigh quotient, for the possible frequencies of waves with prescribed wavelength, and this result served both to establish that the complete problem has solutions in appropriate function classes and to give useful estimates of wave properties.

It seems that the system, like the simpler one without edge constraints, has a fundamental wave mode which can propagate at any given frequency, and which for a given wavelength has the lowest possible frequency. Higher modes have respective cut-off frequencies, which are the frequencies of standing waves independent of distance along the channel, and below which propagation is impossible. For each of the higher modes, the phase velocity is unbounded and the group velocity is zero in the limit as frequency approaches the cutoff value from above. As might be expected, the fundamental mode uniquely has the property that the edges and perpendiculars to them are its only nodal lines: in the cross-section through a longitudinal wave crest, the elevation of the free surface above its undisturbed level is symmetrical about a maximum at the centre, vanishing only at the edges. A less obvious fact emerging from the theory is that at the edges the curvature of the free surface is generally not zero, vanishing only where there is a spanwise nodal line.

For comparison with the experimental results, the theoretical relationship between frequency and wavelength was estimated by means of the Rayleigh–Ritz method, which is particularly amenable in the relevant case of rectangular channels. Taken to two stages of approximation, the method gave results for the fundamental mode in very satisfactory agreement with experiment, and such is the efficiency of the method that even the roughest, first-order estimates seemed to be close to the true values. For example, applied to channels whose breadth, b, is less than ~20 mm and whose depth, h, is not less than b, the following formula gives a good estimate of the fundamental phase velocity, c_1, as a function of wave-number α ($=2\pi$/wavelength):

$$c_1^2(\alpha)=\frac{1.2(g+\gamma\alpha^2)+12\gamma b^{-2}}{\alpha\{\coth(\alpha h)+0.03050(\alpha b)-0.000376(\alpha b)^3\}}$$

Here g is the gravity constant, and γ is the ratio of surface tension to density. This formula is obtainable simply by taking the spanwise distribution of the oscillatory surface displacement to be proportional to $f(x)=(x/b)-(x/b)^2$, where x is the distance from one edge, substituting the function f in the Rayleigh quotient whose absolute minimum expresses $\omega_1^2=\alpha^2c_1^2$, and introducing certain elementary inequalities satisfied by the infinite series whose sum is the denominator of the quotient. The theory proved the formula to be a strict upper bound for $c_1^2(\alpha)$, and it should be particularly close to the true value when αb is much smaller than 1. Over the range of our experiments, the values of $c_1(\alpha)$ predicted by it were at most about 3% in excess of the measured values. Note that in the long-wave limit $\alpha\rightarrow 0$, the estimate is $c_1^2(0)=(1.2g+12\gamma b^{-2})h$, whereas the corresponding well-known result for waves without edge constraints is $c^2(0)=gh$.

Figure 1 presents values of wavelength measured in two channels with different rectangular cross-sections, and also includes theoretical curves for the fundamental mode respective to the two sets of experimental conditions. The Perspex channels, each having a working length of about 1 m and an additional length of about 450 mm with a beach to absorb the waves, were filled exactly to their brims which had been machined smooth. Waves were excited at constant frequency by a small oscillating paddle (made of PTFE) which was driven by an electrodynamic vibrator, and the range of frequencies employed was such that only waves in the fundamental mode would propagate away from the wave-generator. Relative to the original electronic signal driving the motion, variations in the phase of the waves with distance along the channel were monitored by means of a capacitance probe which could be continuously positioned at a fixed height above the water surface, and wavelengths were measured by observing the distances between positions of coincident phase, indicated by Lissajous figures on an oscilloscope.

On the whole the new theoretical results are in good agreement with the experimental observations, although the measured phase velocities tend to be slightly less than those predicted. This discrepancy is in the right direction for it to be accountable in part to the residual error implicit in the use of the Rayleigh–Ritz method, but another possible reason for it can be linked to the difficulty of ensuring a perfectly clean water surface in the channels. Their narrowness made them more or less inaccessible for careful cleaning except by high-speed water jets, and measurements of surface tension *in situ* were impracticable. The water used in the experiments was unable to sustain surface bubbles after being shaken, thus passing a sensitive test for the absence of surface-active contamination, and it was put into the channels by a glass pipette which had been cleaned with chromic acid. As used in the calculations, the value of surface tension was taken to be that for the supply of surface-clean water.

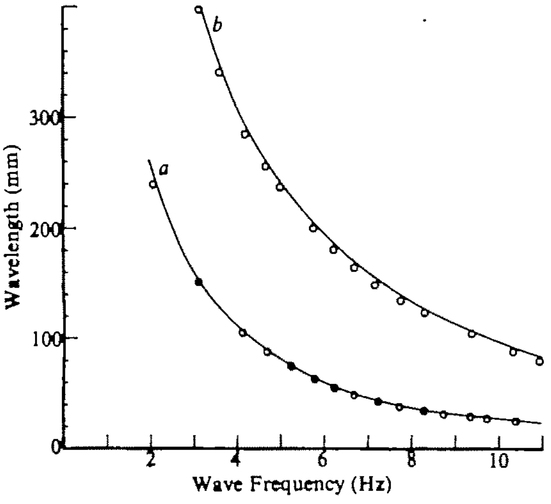

Fig. 1 Measured values of wavelength as a function of frequency, for water waves of small amplitude in two channels: *a*, width 20.3 mm, depth 21.5 mm; *b*, width 4.0 mm, depth 25.0 mm. The solid curves were calculated by the Rayleigh–Ritz method taken to two stages of approximation. Surface tension was estimated to be 72.0 mN m^{-1}.

Contamination subsequently picked up, however, would have had effects consistent with what was actually observed. The discrepancy between measured and predicted wave properties did in fact tend to increase gradually during experimental runs, consistently with a gradual decrease of surface tension.

Capillary wave phenomena depending on edge conditions of this kind seem to have attracted little previous attention and many problems akin to the present one may be suggested for theoretical as well as experimental study. The most promising candidate is perhaps the case of standing waves in closed basins, whether brimful or with fixed contact lines supporting upwards or downwards curving menisci. The nonlinear theory of progressive waves with moderately large amplitudes also poses certain challenging problems. A fuller account of our investigation including a rigorous treatment of the theoretical problem posed by the new class of water waves will be published elsewhere[3].

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SSTs, nitrogen fertiliser and stratospheric ozone

THE threat to stratospheric ozone from supersonic transport (SST) exhaust emissions has been a subject of controversy since Hampson[1] first suggested that significant ozone reductions might be caused by discharged water vapour. Later, after Johnston[2] and Crutzen[3] pointed out that even larger ozone depletions might result from emitted nitrogen oxides, water vapour effects were considered to be relatively unimportant. In the intervening years, predictions of SST ozone modification have evolved with advancing scientific knowledge[4–7]. Using a recently revised model of the stratosphere[8], we report here that a substantial ozone layer enhancement could accompany worldwide SST fleet operations, and that water vapour may be an important factor in SST assessments. We have also found that increased nitrogen fertiliser use might, likewise, enhance the ozone layer, in contrast with previous calculations of ozone reduction[9–11].

Zahniser and Howard[12] have just reported the first direct measurement of the rate coefficient for the reaction

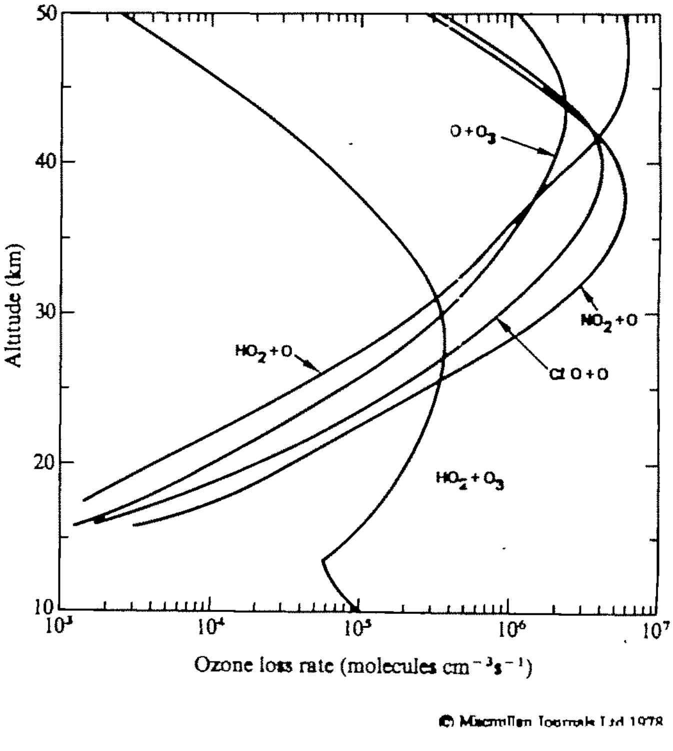
$$HO_2 + O_3 \rightarrow OH + 2O_2 \qquad (1)$$

Their result, $(1.4 \pm 0.4) \times 10^{-14}\ e^{-(580\pm100)/T}$ cm^3 s^{-1} is, at stratospheric temperatures, a factor of 4–6 larger than earlier values, which were based on reaction rate ratios[13]. In the lower stratosphere, where most terrestrial ozone resides, reaction (1) accelerates ozone destruction through a catalytic chemical cycle that also involves the reaction

$$OH + O_3 \rightarrow HO_2 + O_2 \qquad (2)$$

Figure 1 gives the rates of chemical destruction of odd-oxygen (essentially ozone) in the unperturbed stratosphere due to the dominant catalytic reaction cycles involving hydrogen radicals (HO_x = OH and HO_2), nitrogen oxides (NO_x = NO and NO_2), free chlorine gas (Cl_x = Cl and ClO), and the Chapman reactions among the oxygen allotropes. The few chemical processes shown in Fig. 1 represent the most important ozone loss mechanisms in current models of the stratosphere; they are

Fig. 1 Ambient rates of consumption of stratospheric odd-oxygen (ozone) for key catalytic reactions steps.



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responsible for more than 80% of the total ozone loss rate. A complete description of the one-dimensional model used here to make ozone calculations can be found elsewhere[14].

Note in Fig. 1 that HO_x, because of reaction (1), is now the major catalytic agent for ozone loss below 25 km; HO_x also dominates ozone loss above 40 km. Between 25 and 40 km, NO_x has the greatest influence on the ozone loss rate. The Cl_x and Chapman reactions represent important secondary odd-oxygen loss mechanisms (assuming a current atmospheric chlorine level of about 2 parts per billion by volume). The stratospheric ozone content is roughly determined by the balance between loss (as in Fig. 1) and production through O_2 photolysis. The implications for ambient ozone of the new rate constant for reaction (1) are discussed by Whitten *et al.*[15]; some of these implications had been speculated on in earlier publications[6,7].

Shortly before the completion of the Zahniser and Howard[12] study, another major change in aeronomic models occurred when Howard and Evenson[16], and Burrows *et al.*[17] redetermined the rate constant for

$$HO_2+NO \rightarrow OH+NO_2 \qquad (3)$$

The revised rate coefficient, $\sim 8\times10^{-12}\,cm^3\,s^{-1}$ at room temperature, is nearly a factor of 40 greater than previously adopted stratospheric values. The aeronomic effects of reaction (3) are complex[7,8]. Basically, the larger rate constant increases the importance of HO_x relative to NO_x as an ozone-active catalyst, which amplifies the role of reaction (1) in the ozone balance.

Supersonic aircraft cruise at heights well into the stratosphere and release NO_x and water vapour as exhaust products. In a recent detailed assessment of the potential environmental impact of future SSTs, Poppoff *et al.*[8] showed that NO released by SSTs inhibits the normal HO_x catalysis of ozone because reaction (3) diverts HO_2 from reaction (1). Added NO_x also induces faster recombination of HO_x radicals through a reaction of OH with nitric acid, although NO_x regenerates some HO_x (and ozone) through a natural smog mechanism involving methane oxidation[8]. Moreover, NO—which has a fast reaction with ClO analogous to reaction (3)—interferes with chlorine-sensitised ozone destruction. As a result of these coupled interactions, the total content of stratospheric ozone was calculated to increase slightly (a few tenths of a per cent) for modest-sized fleets of SSTs (up to several hundred planes) flying below about 25 km.

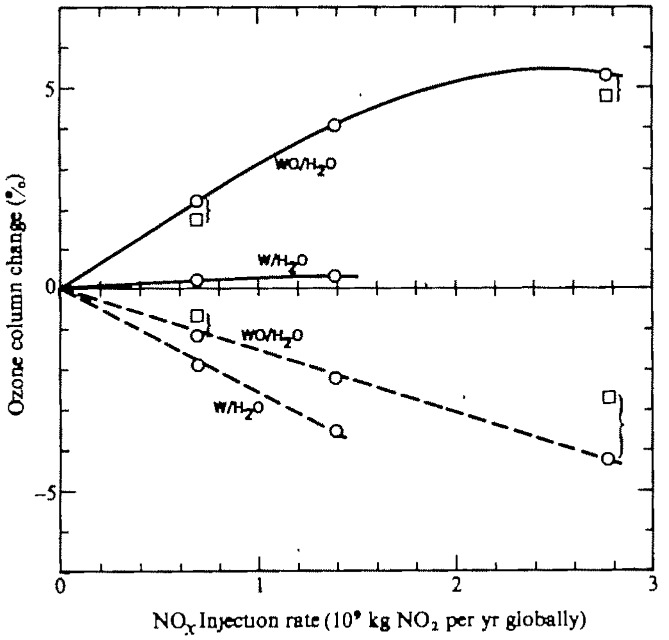

Fig. 2 Steady-state perturbations of the vertical ozone column above 10 km (solid line) and 30 km (dashed line) as a function of the rate of a steady NO_x injection at 20 km. Results are shown both with (W/H_2O) and without (WO/H_2O) simultaneous water vapour release. ○, Values computed using a Wofsy-type (WT) diffusion coefficient; □, Values computed with an alternative (DC) diffusion profile (see text).

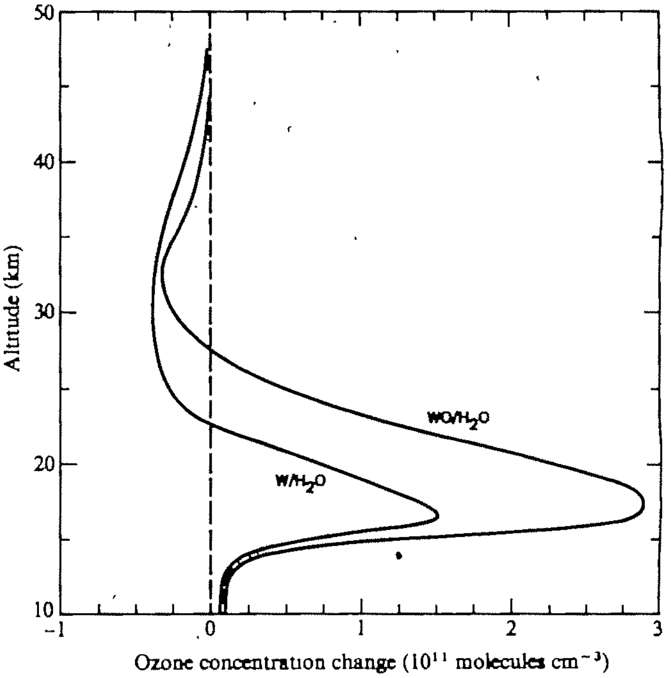

Fig. 3 Calculated steady-state stratospheric ozone concentration changes due to an NO_x injection of 7×10^8 kg NO_2 yr^{-1} (globally averaged) both with and without water vapour emission.

However, Poppoff *et al.*[8] also found that aircraft water vapour emissions may cause significant chemical degradation of ozone because of the following circumstances: (1) H_2O is the major source of stratospheric hydrogen radicals, which now dominate ozone loss throughout most of the stratosphere (see Fig. 1); (2) advanced aircraft technology can (theoretically) suppress NO_x emissions, but H_2O emissions are essentially fixed in proportion to the fuel burned. It has been proposed that ozone depletions due to stratospheric water vapour increases might be largely offset by ozone recovery resulting from H_2O infrared cooling of the air, which slows some of the ozone catalytic reactions[18]. Unfortunately, definitive thermal-feedback calculations related to SST perturbations have yet to be made.

Figure 2 gives our revised estimates of ozone column changes resulting from NO_x injection at 20 km. Two injection cases are considered: (1) NO_x injection only, and (2) simultaneous NO_x and H_2O injection assuming relative engine emission indices of 6 g NO_2 per kg fuel (a design goal), and 1.3 kg H_2O per kg fuel (stoichiometrically determined for jet fuel combustion). Both cases neglect possible thermal feedback effects. Case 2 roughly corresponds to 550 H_2O molecules released with each NO molecule (where NO is taken to be the predominant NO_x species emitted by aircraft engines).

Most of the present calculations were made with the one-dimensional model mentioned earlier using a Wofsy–McElroy type (WT) of eddy diffusion coefficient[19] with the tropopause height adjusted to 13 km (ref. 8); a few cases using a Dickinson–Chang type[20] (DC) are shown for comparison. Note in Fig. 2 that, with regard to the major effects of SSTs on ozone, the differences between diffusion coefficients are not significant. It should also be pointed out that the uncertainty in such model predictions is large[8,20].

According to our latest estimates (Fig. 2), NO_x injection alone could lead to a surprisingly large total ozone enhancement—several per cent or more. In contrast, the ozone content above

30 km would be significantly depleted. Substantial total ozone enhancements are predicted even for the largest NO_x injection rate considered, which corresponds to more than 1,000 advanced aircraft operating on a daily worldwide basis. The reason for these large ozone enhancements can be traced directly to the increased rate constants for reactions (1) and (3). That is, rapid HO_x catalysis of ozone through reaction (1) is strongly suppressed by injected NO through reaction (3), more so in our revised model than in the model used by Poppoff *et al.*[8].

When NO_x and H_2O are injected simultaneously, we still find ozone increases, albeit much smaller ones. Nonetheless, the magnitude of the chemical degradation of ozone by added water vapour is a factor of 2 larger in our updated model because of the faster rate for reaction (1). Calculated ozone changes due to added NO_x and H_2O are quite sensitive to the adopted background chlorine level (2 parts per billion by volume here). Generally, as the chlorine level rises, the ozone enhancement following NO_x injection, and the ozone decrease following H_2O injection, both become larger[8].

Figure 3 shows the predicted ozone concentration changes corresponding to the operation of about 200 aircraft at 20 km. Water vapour chemical reactions are seen to negate in large part the NO_x-induced ozone gains calculated below 25 km. The possible significance to atmospheric temperature and dynamics of such ozone alterations has never been treated satisfactorily.

In the past, the related problem of nitrogen fertiliser and sewage decomposition into free nitrous oxide (N_2O) with subsequent photochemical (NO_x) effects on ozone has been stated in terms of the per cent ozone depletion per unit increase in atmospheric N_2O (refs. 9–11). Using our revised model, however, we calculate that a 50% increase in N_2O abundances could lead to a 2.7% increase in total ozone (above 10 km, for the WT diffusivity). Even so, the ozone content above 30 km would be reduced by more than 5%.

In accordance with our latest estimates of global ozone modification due to stratospheric flight, new research emphasis should be placed on the aeronomical role of water vapour effluence at high altitude, with special regard to its photochemical impact on ozone and infrared effect on air temperature.

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Precambrian solution photochemistry, inverse segregation, and banded iron formations

SOLAR radiation on an early Precambrian sea would generate short-lived excited species near the surface of the water. Many of the species would be powerful oxidising or reducing agents. Despite back-reactions, net reducing power would be lost to the atmosphere. This effect would be the opposite of present day photosynthesis and can be termed 'inverse segregation'. Its possible relevance to banded iron formation is discussed here.

Consider an early Precambrian sea on a summer's day. The atmosphere is neutral (N_2+CO_2) or slightly reducing (+also a little H_2)[1]. High energy UV sunlight (from ~200 nm) thus reaches the seawater which contains Fe^{2+} among other simple inorganic ions. Many of these absorb radiation in the 200–300 nm range, often through charge-transfer-to-solvent transitions[2–6]. As a result numerous short-lived excited species are generated in the upper few centimetres of the water, for example:

$$A^{x-}_{aq} \xrightarrow{h\nu} \cdot A^{(x-1)-} + e^-_{aq} \qquad (1)$$

$$Fe^{2+}_{aq} \xrightarrow{h\nu} (Fe^{2+}_{aq})^* \qquad (2)$$

Many of these are powerful oxidising and reducing agents and may generate others through, for example:

$$e^-_{aq} + H^+ \rightarrow \cdot H \qquad (3)$$

$$(Fe^{2+}_{aq})^* \rightarrow Fe^{3+}_{aq} + OH^- + \cdot H \qquad (4)$$

$$e^-_{aq} + CO_2 \rightarrow \cdot CO_2^- \qquad (5)$$

Exceedingly complex cross reactions and back reactions follow, but not very far below the surface only longer lived oxidised and reduced species are found. These continue to react, often with each other. They cannot fully neutralise each other, however, because during these processes some reduced species have escaped to the atmosphere. In particular H_2, but probably also CO and some of the small organic molecules formed following reactions such as (5) (ref. 6). By contrast oxidising species, particularly radicals[4], have quickly converted Fe^{2+} to Fe^{3+} which precipitates as highly insoluble ferric and ferric–ferrous hydrolysis products. For example:

$$\cdot H + Fe^{2+}_{aq} + H^+ \rightarrow Fe^{3+}_{aq}\downarrow + H_2\uparrow \qquad (6)$$

$$\cdot A^{(x-1)-} + Fe^{2+}_{aq} \rightarrow Fe^{3+}_{aq}\downarrow + A^{x-} \qquad (7)$$

There are, perhaps, still back reactions with reduced species remaining in deeper waters, but net oxidising power remains in the sediments in balance with the reducing power lost to the atmosphere. This effect would be the opposite of present day photosynthesis which puts reduced material in the ground and maintains an oxidising atmosphere. We might call it 'inverse segregation'.

From geological evidence—for Fe^{2+} in ancient seas, and from the presence of easily oxidised detrital minerals in ancient sediments—the atmosphere is widely thought to have been free of oxygen until ~2,000 Myr ago[7,8]. That was the great period of ferric sedimentation in the form of the massive and still problematical banded iron formations (BIFs)[7–10]. Typically these consist of alternate iron-rich and iron-poor layers within siliceous sedimentary rocks. The layers are generally from 0.5–3 cm thick with, frequently, 'microbands' on the mm scale or less that may, nevertheless, extend for hundreds of kilometres: the microbands have been attributed to an annual even outfall from large bodies of water[11,12]. Could inverse segregation explain these largely oxidised deposits?

It might account qualitatively for the general history of BIF sedimentation. The record starts with the oldest known sediments at 3,800 Myr ago[13,14]. As well as carbonates and BIFs, these rocks contain only very small amounts of graphite[14] suggesting that a strongly reducing methane-laden atmosphere, if it ever existed, had passed away by this time[15]. As the evolving atmosphere became still less reducing, through a continuing loss of hydrogen to space, together with diminishing volcanic sources[1]. reduced species derived from the atmosphere would less often reconvert oxidised precipitates before they reached the sediments. Hence, when the atmosphere was just neutral, inverse segregation should have been at its most effective, corresponding, perhaps, to the greatest period of the BIFs just before they became rare at ~2,000 Myr ago[8]. In the subsequent faintly oxidising regime (until ~700 Myr ago[7]) BIFs could still form, but only in special situations where water masses were fed by submarine sources of Fe^{2+} (refs 8, 10). After that, with substantial photosynthetic oxygen, an ozone screen would have prevented further large scale photoprecipitation. (The BIF record finally peters out at ~800–600 Myr ago[16].)

Quantitatively the idea also seems feasible. If we take the present solar spectral irradiance curve between 200 and 300 nm (ref. 17) and if we assume also a transparent atmosphere in this range, then about 0.03 einsteins yr^{-1} could have struck an average cm^2 of sea surface. If each photon lead to the deposition of one Fe^{3+} ion, the sedimentation rate would be ~1,700 $mg\ cm^{-2}\ yr^{-1}$. This can be compared with the range of between 8 and 43 $mg\ cm^{-2}$ for total iron (some of it Fe^{2+}) found in microbands attributed to an annual outfall[12]. Some set of photoredox processes with an overall quantum yield of ~0.005–0.02 would seem to be needed.

Quantum yields of this order are common enough for laboratory photoredox processes. One reaction that is attractive for its simplicity is the well known evolution of H_2 from acid ferrous solutions[4,18,19]; formally:

$$Fe^{2+} + H^+ \xrightarrow{h\nu} Fe^{3+} + \tfrac{1}{2}H_2\uparrow \qquad (8)$$

Using a low pressure mercury lamp with a filter to cut out radiation below 200 nm, initial quantum yields of about 0.12–0.07 were obtained over the *p*H range 1–3 with 0.02M $FeSO_4$ (ref. 4). This may seem too acid for natural waters. It might be objected also that quantum yields fall off as Fe^{3+} builds up[4]. However, the hydrolysis of Fe^{3+}:

$$Fe^{3+} + 2H_2O \rightarrow FeOOH\downarrow + 3H^+ \qquad (9)$$

makes more protons than reaction (8) uses, suggesting possible autocatalysis in surface waters becoming more acid than the sea generally. Reaction (9) should also remove Fe^{3+} from the photoreaction zone by precipitation. (A fresh 0.01M solution of $FeCl_3$ has a *p*H of ~2.3 which after 2 days falls to about 1.8 by which time visible precipitation has set in [20].)

Getoff's experiments too are suggestive: organic molecules are formed by UV irradiation of CO_2-saturated solutions in the *p*H range 3.5–8.7 (ref. 6).

Even with the minimum model represented by equations (8) and (9) we can see that particular conditions of Precambrian seas, such as general *p*H, rates of mixing, and presence of buffers, could have been critical. And there are many other unknowns that could have greatly complicated the situation: traces of ions photoactive at longer wavelengths; a steady-state mix of organic molecules as possible scavengers[2,5] or photosensitisers[18]; or colloidal mineral crystallites allowing surface or solid-state photoreactions[21]. One conclusion is independent of very detailed knowledge: some photoredox processes would surely have been occurring in early Precambrian waters with, sometimes, the formation of volatile reduced species: if the atmosphere remained a sink for these (ultimately through loss of hydrogen to space) then so long as Fe^{2+} was there to prevent the escape from the seas of the corresponding oxidised species, inverse segregation would be a predictable outcome. Whether the banded iron formations are to be explained mainly or partially in such terms is another matter—but this non-biological light-driven mechanism seems qualitatively and quantitatively feasible.

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Stable isotopic compositions of early Archaean sulphate deposits of probable evaporitic and volcanogenic origins

SIGNIFICANT Archaean bedded sulphate deposits have been described only from the Pilbara Block (Western Australia)[1,2], the Barberton Mountainland (South Africa)[3–6] and southern India[7]. These are all barite deposits, but there is petrographic evidence suggesting replacement of original evaporitic gypsum in the North Pole (Pilbara) and Barberton deposits. This contrasts with the apparent volcanogenic deposition of barite in the relatively sulphide-rich Big Stubby prospect (Pilbara). This letter summarises the petrographic evidence, concentrating on recent data from North Pole, and reports stable isotopic results for barite and associated sulphides which provide corroborative evidence for the environments of formation of these deposits. Some implications concerning the early hydrosphere, atmosphere and biosphere are outlined.

The North Pole deposits[8,9] (21°07′ S, 119°28′ E) lie within a ~30-m thick fossiliferous[10] sequence consisting of laminated chert, silicified arenite and conglomerate, between slightly metamorphosed mafic and ultramafic volcanics of the Warrawoona Group[11]. Stratigraphic correlations suggest the North Pole sequence is of similar age to the ~3.45-Gyr-old[12] felsic volcanics of the Duffer Formation, and this is supported by a ~3.4-Gyr 'model age' for North Pole galena (J. Richards, personal communication). Various structures and textures suggest that the bedded barite and chert replaced an original evaporitic gypsum–carbonate sequence[8,9]. The barite beds are composed of bottom-nucleated crystal groups, 5–20 cm in length, radiating across bedding (Fig. 1). These strongly resemble 'cavoli' and grass-like evaporitive gypsum structures[13], and they contrast markedly with barite forms from both volcanic–exhalative and primary sedimentary deposits[14,15]. Some North Pole barite crystals have swallow-tail twins, and where it has been possible to measure interfacial angles of undeformed single

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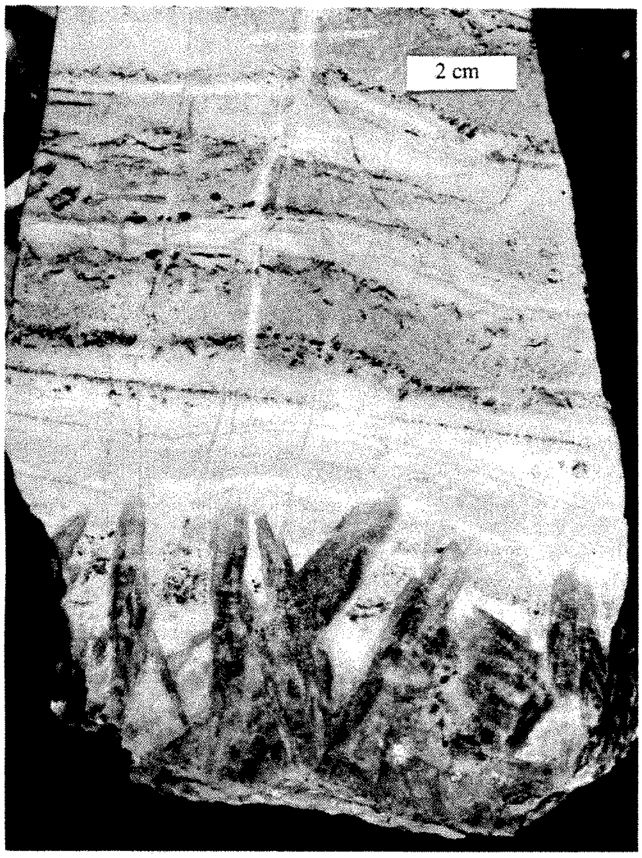

Fig. 1 Section of stratiform barite–chert horizon from North Pole showing large barite pseudomorphs after gypsum with overlying laminated chert (possibly stromatolitic). Universal stage measurements confirm that undeformed barite crystals have gypsum interfacial angles; mean angles for five measured crystals are $110 \Lambda 1\bar{1}0 = 68°12' \pm 0°50'$ and $\bar{1}10 \Lambda \bar{1}11 = 59°00' \pm 1°00'$. Most bedded barite crystals contain irregular domains in which barite is slightly misorientated with respect to adjacent domains and to the length of the crystal.

barite crystals, these have indicated crystal faces typical of gypsum but not recorded for barite (Fig. 1). Thus at least some barite is demonstrably pseudomorphing gypsum. Synsedimentary formation of the precursor gypsum is indicated by eroded crystals tops, and sediment drapes and fills between crystals. Intraformational conglomerates containing clasts of bedded barite attest to early lithification of precursor gypsum, if not early baritisation.

Clear, cryptocrystalline silica pseudomorphs after single or twinned gypsum crystals, commonly at high angles to bedding, occur in laminated, fine-grained, grey chert (Fig. 2). Ghost laminae within some of these silica pseudomorphs suggest a diagenetic origin for the crystals. Silica pseudomorphs after cubic minerals, and cubic voids, have also been observed in some chert beds, and a silicified hopper-shaped crystal occurs in one thin section. However, as is also the case in some younger evaporites, there is no evidence for the former presence of abundant halite at the North Pole.

That chert replaced carbonate sediments at North Pole is indicated by relic carbonate which is partially replaced by silica, stylolitised dolomite rhombs, and rhombic voids. Peritidal deposition of chert precursors is implied by laminated (possibly stromatolitic) bedding, graded- and cross-bedding, scour-and-fill structures, intraclast and edgewise conglomerates, ooid grainstones, ripple marks and probable desiccation features such as curved intraclasts and possible mud cracks. Thus depositional environments deduced independently from interlayered barite and chert are entirely consistent.

Minor bedded pyrite and sphalerite occur near the extremities of the North Pole barite and there are small galena pods within zones of recrystallised barite. Fluid inclusions in barite contain liquid CO_2, water and traces of H_2S.

The North Pole deposits contrast with the similarly ancient Big Stubby baritic–sulphide deposit in the Duffer Formation, some 40 km to the east. Published descriptions of Big Stubby[16,17] emphasise its geological, mineralogical and textural similarities to Kuroko-type volcanogenic deposits.

The barite near Barberton[3,4] (25°45′ S, 31°00′ E) is in the slightly metamorphosed, fossiliferous[18] Swaziland Supergroup. It is interbedded with carbonated felsic metavolcanics in a cherty sequence at the top of the ~3.45 Gyr (ref. 19) Onverwacht Group and in chert and siltstone of the overlying ~3.2 Gyr (refs 4, 19) Fig Tree Group. As at North Pole, much of the barite characteristically grew subperpendicular to bedding. It has been noted that it generally resembles evaporites in texture[4,10], and some small barite crystals in chert pseudomorph gypsum[20]. Thus, at least some Barberton barite probably formed in the same way as North Pole barite.

The isotopic results are shown in Fig. 3. δ^{18}O values for barite from North Pole and Barberton are both within the range +4.5–+10.1‰, except for a single Barberton value of +13.3‰. These are the first recorded δ^{18}O values for Archaean sulphates and they are, on average, less positive than for Recent evaporitic gypsum (~+9 ± 13‰)[21], possibly supporting the proposal that the Archaean hydrosphere was ^{16}O-enriched relative to younger waters (see ref. 22).

The δ^{34}S values for barite from widespread locations at the North Pole (average +3.6‰, s. d. 0.5‰) and the Barberton deposits (average +3.8‰, s. d. 0.4‰) are remarkably consistent. They are similar to the results obtained by Perry *et al.*[2,5] on small sample populations from each area and less variable than the results of Vinogradov *et al.*[6] for all barite morphologies in the Fig Tree Series (average +5.0‰, s. d. 1.5‰). Individual post-Archaean evaporitic sulphate deposits also have uniform sulphur isotope composition, but their δ^{34}S values are characteristically $\geq +10$‰ (ref. 23); apparently they commonly reflect the isotopic composition of contemporaneous seawater SO_4^{2-}, with δ^{34}S values of sulphate minerals being a maximum of 2‰ heaver than SO_4^{2-} in the parent brine[23]. The purportedly volcanogenic barite at Big Stubby has markedly higher δ^{34}S values than barite from North Pole and Barberton, and is evidently not reflecting the $\delta^{34}S_{SO_4^{2-}}$ values in the contemporaneous hydrosphere.

The δ^{34}S values for sulphides from North Pole and from sedimentary rocks penecontemporaneous with the Barberton

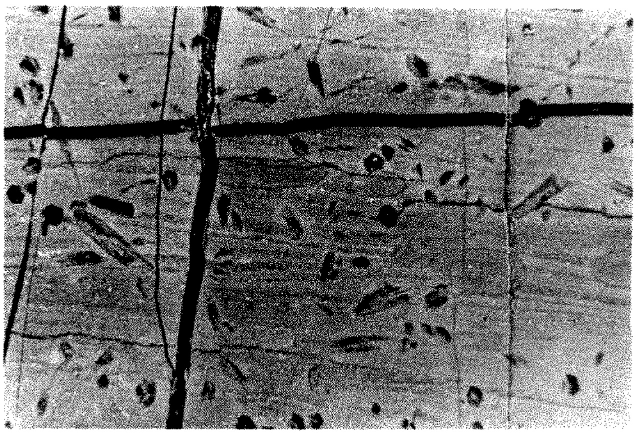

Fig. 2 Photomicrograph of laminated chert (light grey) from North Pole containing well formed silica pseudomorphs after gypsum (medium grey). Pseudomorphs show characteristic interfacial angles for gypsum. Mean angles for 12 crystals are $110 \Lambda 1\bar{1}0 = 68°48' \pm 1°18'$, $110 \Lambda 010 = 56°00' \pm 0°33'$ and $\bar{1}10 \Lambda \bar{1}11 = 59°00' \pm 0°55'$. Field of view 3.5 × 2.4 cm.

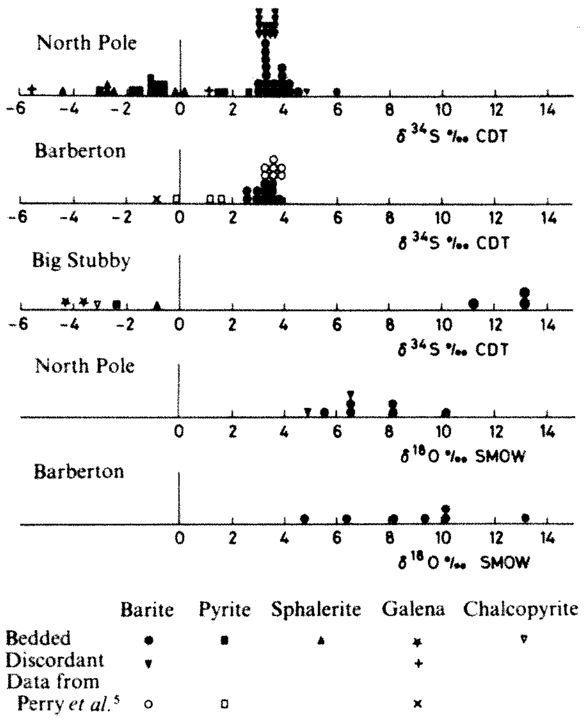

Fig. 3 Stable isotope data. The results from North Pole and Barberton are similar, and they corroborate petrographic evidence for sedimentary–evaporative deposition. Discordant barite at North Pole formed by remobilisation of bedded barite as a result of tectonic doming[8,9]. The contrasting $\delta^{34}S$ pattern at Big Stubby supports textural, mineralogical and geological evidence[16] for its volcanogenic origin. Weighted mean $\delta^{34}S_{\Sigma S}$ values indicate sulphur of juvenile derivation in each case.

barite deposits average −0.9‰ (s. d. 1.5‰), and Big Stubby sulphides have a slightly more negative average $\delta^{34}S$ value. These are similar to values in sulphate-free, Archaean volcanogenic sulphide deposits[24], but differ from younger volcanogenic sulphide deposits which generally do not have $\delta^{34}S$ values close to 0‰ (ref. 25).

The weighted mean $\delta^{34}S$ values for the essentially monomineralic barite deposits at North Pole and Barberton, and for the sulphide-rich Big Stubby deposit, are similar. In each case the $\delta^{34}S_{\Sigma S}$ in the depositional waters must have been close to 0‰, implying sulphur of juvenile origin. The $\Delta^{34}S$ (barite–sulphide) values of $\sim +15‰$ at Big Stubby are comparable with those in the younger volcano-exhalative deposits[25]. In contrast, the much smaller $\Delta^{34}S$ (barite–sulphide) values at North Pole and Barberton (average $< +5‰$) show no evidence of approach to isotopic equilibrium at hydrothermal or surface temperatures[26]. Ascending juvenile fluids could not have contained $SO_4^{2-} \gg H_2S$, HS^- because sub-surface reactions would have favoured the reduced species. Therefore, the isotopic results indicate that sulphates at North Pole and Barberton could not have been deposited directly from exhalations. These sulphates must have formed largely as a consequence of surficial oxidation of reduced sulphur which was introduced into the hydrosphere by exhalative activity. Oxidation could have been effected by blue-green algae or sulphur bacteria (microfossils are present[10,18]), although the importance of oxygen production by photolysis of water vapour[27] cannot be ruled out. Oxidation of reduced sulphur is a rapid non-equilibrium process which does not lead to significant isotopic fractionation.

The clear evidence for precursor gypsum at North Pole and Barberton argues against deposition of major proportions of primary barite, as proposed by Perry *et al*[5]. The only model satisfying both isotopic and petrographic constraints is one in which the high concentrations of isotopically homogeneous SO_4^{2-} required for sedimentary gypsum deposition built up in restricted basins as a result of evaporation and recharge from external sources.

Baritisation of the gypsiferous sediments was presumably accompanied by precipitation of additional barite as the Ba^{2+}-bearing fluids reacted with residual SO_4^{2-} in basin and interstitial waters. Barite produced by these processes would have $\delta^{34}S$ values similar to precursor gypsum. The origin of the Ba^{2+} is uncertain, but it may have been derived from exhalations related to felsic volcanism (Duffer Formation), or from diagenetic-burial metamorphic reactions.

The minor amounts of sulphides with the barite probably formed from exhalative sulphur which was not oxidised in the depositional basins: the presence of H_2S in fluid inclusions may be significant in this respect. Had they formed by bacterial reduction in SO_4^{2-}-rich evaporative environments, the sulphides would have had wide ranges of $\delta^{34}S$ values, most being highly negative relative to $\delta^{34}S$ values of sulphate. Except for the ~2.7-Gyr BP Michipicoten iron formation[28], available isotope data for Archaean sulphide mineralisation have not produced evidence for bacterial SO_4^{2-} reduction[24].

The occurrence of gypsum and barite does not require an oxygenated atmosphere in the early Archaean; this would only have existed if the oxygen 'sinks' (such as, H_2S, CH_4, Fe^{2+}) in the hydrosphere and atmosphere were saturated. The sulphate-free volcanogenic sulphides in other, apparently deeper-water Archaean sequences provide evidence that the hydrosphere was not everywhere oxidised. They suggest either the presence of reduced waters under a surficial oxidised zone[29,30] or the existence of separate seas with different degrees of oxidation.

The Archaean scenario envisaged here is one in which: (1) juvenile, sulphurous exhalations (H_2S, $HS^- \gg SO_4^{2-}$) were being added to the hydrosphere and atmosphere; (2) there was local oxidation of reduced sulphur by microbes (or photolysis), but oxygen was not necessarily a stable component of the atmosphere; (3) bedded sulphate deposits formed in shallow-water, evaporitic environments; (4) relatively small, baritic sulphide deposits precipitated directly as a result of volcano–exhalative activity; (5) sulphate-free, exhalative sulphide mineralisation formed in deeper water and/or lower f_{O_2} environments; and (6) bacterial SO_4^{2-} reduction was absent, or not of widespread significance.

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Metal-enriched sediments from the TAG Hydrothermal Field

A SERIES of sediment samples from the TAG Hydrothermal Field[1] have been collected and analysed for transition metals, and for uranium and thorium isotopes. We show here that metal enrichment of sediment samples is ubiquitous, but geographically variable. Non-detrital metal accumulation rates in one core are Fe and Mn 15.0 and 0.7 mg cm^{-2} 10^{-3} yr; and Ni, 180; Co, 93; Cu, 134; and Cr, 148 μg cm^{-2} 10^{-3}yr. The hydrothermal field is located on the eastern wall of the Mid-Atlantic Ridge Rift Valley at 26°N and covers ~100 km^2. It is characterised by extensive deposits of thick, rapidly growing MnO_2 (ref. 2) and by a magnetic low in the axial anomaly[1].

Figure 1 shows that the sediment thickness is quite variable even over a small area of the valley floor. Core 4A (132 cm of sediment) and core 4G (15 cm of sediment) both terminated with basaltic glass, and a core from site 4C recovered glass only, with no overlying sediment. A study of bottom photographs of this section of the median valley[3] shows ripple marked sand and other evidence of the strong bottom currents responsible for sweeping sediment off some portions of the valley floor into sediment ponds of uneven distribution.

Three types of sediment were collected during the 1973 cruise of the Discoverer: (1) samples 4A, 4B and 4H are cores of ponded carbonate ooze sediment in the median valley; (2) $CaCO_3$-rich clay was dredged from the top of a small terrace (dredge site 2A); and (3) sediment interstitial to and covering boulders in the talus was recovered from dredge sites 2A and 3A. This material was less than 5% carbonate and consisted largely of altered basalt debris.

Chemical analyses were made on the sediment samples by atomic absorption spectrophotometry. The results are given in Table 1 along with metal-to-aluminium ratios for average North Atlantic pelagic clay[4–11] for comparison. The metal-to-aluminium ratios for the average North Atlantic clay is considered to be characteristic of the detrital component of the sediment on which hydrothermal additions are superimposed. Note that none of the samples analysed are very highly enriched in metals compared to the average North Atlantic pelagic clay values. The highest Mn/Al ratios were observed in one of the samples from Dredge Site 2A and the $CaCO_3$-rich terrace sediment, but the maximum enrichment of Mn relative to the average North Atlantic clay Mn/Al value was only about 7, in contrast to enrichments of 100 or higher found on the East Pacific Rise[12]. The presence of a large deposit of Mn oxide found in the nearby hydrothermal field[2] clearly suggests a significant flux of Mn into seawater in this general vicinity. Scott *et al.*[2] have measured the accumulation rate of the Mn oxide deposit and found it to be 100–200 mm Myr^{-1} or 2 orders of magnitude faster than typical deep sea ferromanganese deposits. It is surprising that so little of the Mn is deposited in the adjacent sediments.

Iron-to-aluminium ratios in the TAG sediments showed even less pronounced enrichment than the Mn/Al ratios. The average Fe/Al ratio for all samples analysed is 0.89 in comparison with the average North Atlantic pelagic clay ratio of 0.56 (Table 1). Most of the Fe in these sediments is detrital in origin. Betzer *et al.*[13] have found much higher Fe/Al and Mn/Al ratios on a total sample basis for the suspended matter sampled from near bottom water over the ridge crest than for similar particles sampled to either side of the ridge crest. Some of the Fe and Mn in the hydrothermal fluids are probably precipitating in the water column after they enter the ocean. They are diluted in the underlying sediment by biogenic and detrital material being deposited at the same site, and are undoubtedly also being wafted away from the area by currents. The nearly total absence of Fe in the massive hydrothermal MnO_2 deposit[2] (Fig. 1) suggests that the Fe may be removed as pyrite[14] or other minerals before the hydrothermal fluids debouch onto the sea floor from that vent. The variability in metal enrichments between the massive MnO_2 deposits and the metal enriched sediments indicates variability in the chemistry of the hydrothermal fluids in both space and time.

The metal-to-aluminium ratios of samples from core 4A show that the core is somewhat enriched throughout its length in Fe, Mn, Ni, Cu, Co and Cr with respect to average North Atlantic pelagic clay (Table 1). The Mn content of the 30–32 cm interval falls below the average pelagic clay value, but this is probably the result of dilution by basaltic glass fragments at that depth that were not removed from the sediment before analysis. The Co and Cu values are very strongly correlated with Mn content, showing correlation coefficients of +0.95 and +0.97 respectively. These metals may be concentrated from seawater by precipitating hydrothermal Mn, or they may also be contributed by the hydrothermal solutions themselves. Nickel and Cr in the core are not correlated with Fe, nor with Mn, nor with each other. The factors controlling their distribution cannot be determined without further investigation.

Metal enrichment studies of ridge crest sediments based only on metal-to-aluminium ratios omit a very important parameter—time. The ratios observed in the sediment reflect not only the intensity of the metal source, but also all of the other processes operating to dilute the metals being added by ridge crest processes. An estimate of the rate at which the metals are added compared to analogous rates for non-metalliferous sediments avoids this problem.

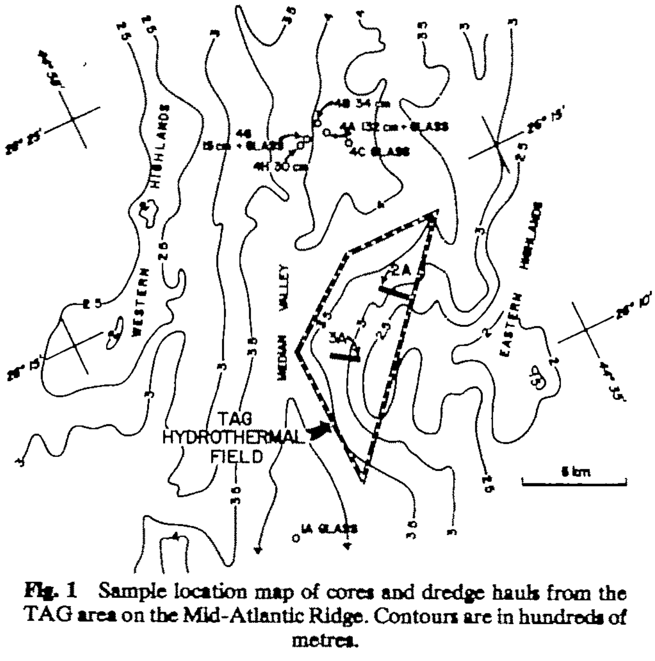

Fig. 1 Sample location map of cores and dredge hauls from the TAG area on the Mid-Atlantic Ridge. Contours are in hundreds of metres.

Table 1 Sediment analyses ($CaCO_3$-free basis)

	Fe (%)	Mn (%)	Al (%)	Ni (p.p.m.)	Co (p.p.m.)	Cu (p.p.m.)	Cr (p.p.m.)	Fe/Al	Mn/Al	Ni/Al $\times 10^3$	Co/Al $\times 10^3$	Cu/Al $\times 10^3$	Cr/Al $\times 10^3$	% $CaCO_3$	Porosity (ml cm^{-3})
2A8-2	6.34	0.145	7.40	128	54.0	47.7	254	0.86	0.0196	1.73	0.730	0.645	3.43	nd*	—
2A8-3	4.26	0.226	2.97	123	59.6	269	242	1.43	0.0761	4.14	2.01	9.06	8.15	nd	—
2A8-4	6.97	1.19	6.63	164	58.2	88.5	342	1.05	0.179	2.47	0.878	1.33	5.16	nd	—
Dredge Site 3A†															
3A2-1	7.47	0.146	8.04	135	61.3	91.3	370	0.93	0.0181	1.68	0.762	1.14	4.60	nd	—
3A2-2	3.15	0.096	3.45	76	42.6	61.2	94	0.91	0.0278	2.20	1.23	1.77	2.72	nd	—
3A2-3	3.73	0.105	3.79	92	35.7	74.0	236	0.98	0.0277	2.43	0.942	1.95	6.23	nd	—
Terrace Sediment‡															
2A8-5	2.90	0.932	3.15	341	140	150	126	0.92	0.296	10.8	4.44	4.76	4.00	77.0	—
Core Tops															
4B02	5.98	0.349	6.74	147	105	166	230	0.89	0.0539	2.18	1.62	2.46	3.41	60.3	—
4G04	5.50	0.528	6.10	204	150	197	208	0.90	0.0866	3.34	2.46	3.23	3.41	72.8	—
4H08	4.89	0.343	5.58	178	108	179	212	0.88	0.0615	3.19	1.94	3.21	3.80	61.2	—
Core 4A															
0–4 cm	5.32	0.407	6.19	218	138	212	244	0.86	0.0658	3.52	2.22	3.41	3.94	67.3	0.73
4–8	5.15	0.421	4.98	198	140	221	204	1.03	0.0845	3.98	2.81	4.44	4.10	69.6	0.74
8–14	3.90	0.478	4.99	229	161	247	180	0.78	0.0958	4.59	3.23	4.95	3.61	73.9	0.75
14–18	3.49	0.419	5.64	212	154	225	169	0.62	0.0743	3.76	2.73	3.99	3.00	72.8	0.71
18–22	4.08	0.472	5.98	235	164	240	184	0.68	0.0784	4.23	2.74	4.01	3.08	75.0	0.73
22–26	4.93	0.441	6.49	206	156	230	190	0.76	0.0680	3.17	2.40	3.54	2.92	73.1	0.75
26–30	5.33	0.254	6.94	261	87.6	146	232	0.77	0.0366	3.76	1.26	2.10	3.34	46.5	0.75
30–31§	4.68	0.205	7.04	276	88.9	442	220	0.65	0.0291	3.92	1.26	6.28	3.12	44.6	—
31–32§	5.53	0.184	8.39	254	81.6	119	256	0.66	0.0219	3.03	0.973	1.42	3.05	31.8	—
32–33§	5.35	0.257	5.01	286	87.8	148	228	1.07	0.0513	5.71	1.75	2.95	4.55	48.6	—
33–37	5.84	0.333	6.12	312	99.9	190	125	0.95	0.0544	5.10	1.63	3.10	2.04	55.9	0.79
120–127	5.86	0.296	6.53	134	79.9	146	212	0.90	0.0453	2.05	1.22	2.24	3.25	51.6	0.77
Avg. N Atlantic pelagic clay[1–11]	5.0	0.4	9.0	79	39	115	80	0.56	0.04	0.9	0.4	1.3	0.9	—	—
Mid-Atlantic Ridge 45°N[7]	9.6	0.5	5.3	214	—	399	128	1.8	0.1	4.0	—	7.5	2.4	—	—
East Pacific Rise[12]	18.0	6.0	0.5	430	105	730	55	36	12	86	21	146	11	—	—
Avg. Pacific pelagic clay[4]	5.1	0.9	8.3	300	110	400	78	0.61	0.11	3.6	1.3	4.8	0.9	—	—

* Negligible, not determined. † Material interstitial to talus blocks ‡ $CaCO_3$-rich sediment from step-fault terrace § Most glass shards removed as >0.088 mm fraction. Porosity measurements were made by John Morse

Table 2 Radiometric data from TAG sediment core 4A

Sample depth (m)	U (p.p.m.)	$^{234}U/^{238}U$	Th (p.p.m.)	^{230}Th d(pm g^{-1})	$^{230}Th/^{232}Th$	$^{230}Th \times s$ d(pm g^{-1})
0–4	0.58 ± 0.03	1.12 ± 0.07	1.38 ± 0.06	14.06 ± 0.40	10.22 ± 0.38	13.58 ± 0.40
18–22	0.75 ± 0.03	1.40 ± 0.07	4.07 ± 0.11	11.60 ± 0.18	11.58 ± 0.27	10.82 ± 0.18
22–26	0.83 ± 0.03	1.37 ± 0.05	6.47 ± 0.44	11.83 ± 0.50	7.43 ± 0.45	10.99 ± 0.50
26–30	0.59 ± 0.03	1.20 ± 0.07	4.55 ± 0.16	10.88 ± 0.24	9.72 ± 0.30	10.37 ± 0.24
33–37	0.73 ± 0.01	1.00 ± 0.02	3.55 ± 0.23	11.32 ± 0.37	12.93 ± 0.77	10.79 ± 0.37
41–50	0.64 ± 0.03	1.26 ± 0.08	6.82 ± 0.19	11.72 ± 0.22	6.98 ± 0.16	11.11 ± 0.22
57–70	0.36 ± 0.04	0.75 ± 0.11	2.51 ± 0.08	8.09 ± 0.11	13.12 ± 0.41	7.89 ± 0.11
120–127	0.65 ± 0.01	1.17 ± 0.03	4.04 ± 0.24	8.27 ± 0.27	8.31 ± 0.46	7.71 ± 0.27

1 s.d. errors from counting statistics are listed.

Table 3 Metal accumulation rates for core 4A

	Fe	Mn	Al	Ni	Co	Cu	Cr	$CaCO_3$-free sedimentation rate (mm kyr^{-1})
		(mg cm^{-2} kyr^{-1})				(μg cm^{-2} kyr^{-1})		
Core 4A, whole sediment	49.5	3.47	61.9	235.0	120.0	214.0	204.0	6.8
Core 4A, detrital fraction	34.5	2.78	61.9	54.8	27.0	80.1	55.6	—
Core 4A, nondetrital* fraction	15.0	0.69	0	180	93	134	148	—
Average N. Atlantic pelagic clay	8.7	0.7	15.6	13.8	6.8	20.2	14.0	2.5
East Pacific Rise a	82.0	28.0	0.61	160.0	—	—	—	0.5
East Pacific Rise b	58	18	3	153	47	254	21	5.3

* The accumulation rate for the non-detrital fraction of Core 4A was calculated by subtracting a detrital metal component based on the detrital metal-to-aluminium ratio (average North Atlantic pelagic clay) and the accumulation rate of aluminium in Core 4A.

The sedimentation rate of core 4A was measured by means of the ^{230}Th excess method (Table 2). The rate at which this sediment is accumulating is ~1.8 cm kyr^{-1}. This rate is consistent with Mid-Atlantic Ridge sedimentation rates measured by other workers[15–17]. Note in Table 2 that ^{234}U/^{238}U activity ratios >1 exist over the whole length of the core, in some cases beyond corrections for 3 s.d. of counting statistics. Post depositional hydrothermal additions of uranium to ridge crest sediments have been previously noted[18–21]. The same interpretation seems reasonable for these data. Addition of ^{234}U-rich uranium would distort the accumulation rate towards a lower value.

Metal accumulation rates have been calculated for core 4A on the basis of average metal concentrations and porosity (Table 1) and the measured sediment accumulation rate of 1.8 cm kyr^{-1} and are listed in Table 3. The Al content of marine sediments is commonly used as an index of the detrital component. We calculated the metal accumulation rates for the detrital component of core 4A using the average Al content of 4A (Table 1), the sediment accumulation rate of 4A, and the metal-to-aluminum ratios for average North Atlantic pelagic clay (Table 1). These values were subtracted from the whole sediment values, yielding the non-detrital accumulation rates listed in Table 3. These rates include only material from non-detrital sources.

The non-detrital metal accumulation rates (Table 3) show that Fe, Mn and the trace metals are being added to sediment in the TAG area at rates that are well above the background values for the North Atlantic Ocean. Iron and manganese accumulation rates on the East Pacific Rise[22–24] are much greater than at this site on the Mid-Atlantic Ridge. However, the Ni, Co, Cu, and Cr accumulation rates are conspicuously high in comparison with East Pacific Rise data. The differences observed in sediments from the two active ridge areas may be related to water circulation differences in the hydrothermal systems of fast and slow spreading ridges[25]. Note also that metal enrichment in TAG area sediments may be higher than average for hydrothermal areas on the Mid-Atlantic Ridge. Sediments from the FAMOUS area at 37° N were found to be accumulating metals at much slower rates than those reported here[18].

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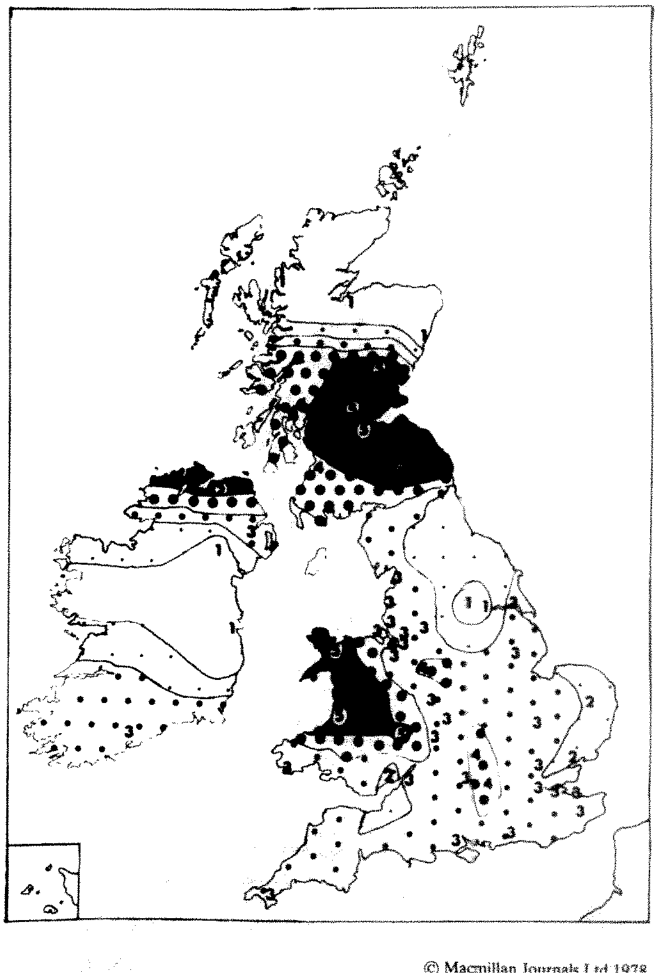
A survey of ozone levels in the British Isles using indicator plants

THE occurrence of elevated levels of photochemically-produced ozone in the British Isles has been reported by several workers[1–4]. Such elevated concentrations are not restricted to urban areas, but can occur far from sources of pollution[1,3,5,6]. Most of the measurements of ozone have been made in South-east England; there has been no systematic attempt to study its distribution over the whole country. We report here results of a nationwide survey of ozone concentrations, using the appearance of visible injury on the leaves of a sensitive plant species as an indicator of the presence of the pollutant.

Fig. 1 The distribution of leaf injury, derived by computer interpolation from the mean values of weekly leaf injury index at 53 sites for the period 13 June–5 September 1977. Five classes (denoted by increasing degrees of shading) are distinguished, covering the following ranges of values: 1, 0·01–0.54%; 2, 0.54–1.06%; 3, 1.06–1.59%; 4, 1.59–2.11%; 5, 2.11–2.64%.



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The plant used in this study was *Nicotiana tabacum* cv. Bel-W3. This cultivar has a threshold for the appearance of visible leaf injury at 0.04–0.05 p.p.m. ozone (the maximum concentration in which ozone normally occurs naturally), and the extent of leaf injury has been shown to be related to the amount of ozone to which the plant has been exposed[7–9]. The plants normally were grown in heated glasshouses in standard composts and then 'hardened off' for one week in an unheated glasshouse, before being placed in the field. Their age at this time was 8 weeks. The plants remained in the field for 4 weeks, and were then replaced by a fresh batch of 8-week-old plants. Six replicate plants were exposed at each site. The exposure sites chosen were open, and at a suitable distance from any local source of other pollutants. Wind protection was provided.

The percentage of the area of each leaf covered by typical ozone-induced lesions was estimated visually at each site at weekly intervals. From these data, the change in the mean percentage of leaf area injured (which we term the leaf injury index) was calculated for each week. Photographs and leaf samples were provided to help in identification of ozone damage, while at many sites, plants of an ozone-resistant cultivar, Bel-B, which showed little or no ozone damage, were placed alongside the test plants. Leaves were sent to us to confirm the identification of damage, and to determine the accuracy of damage assessment by comparing the visual estimates with objective estimates obtained using a grid placed over the leaf.

Figure 1 shows the distribution of leaf injury. Because of an absence of data, little importance can be attached to the interpolated values in certain areas (for example, the sharp changes in injury around Berwick). However, it is encouraging that values for neighbouring sites were usually in agreement. Ozone is produced by a series of photochemical reactions from nitrogen oxides and reactive hydrocarbons. The major source of these pollutants is motor vehicle exhausts, and thus high concentrations of ozone, and large amounts of leaf injury, might be expected to occur in areas of high population density. Yet in 1977, the amount of leaf injury in certain urban areas was relatively low, while it was high in some rural areas, such as North Wales. Only in northern Scotland was no leaf damage found.

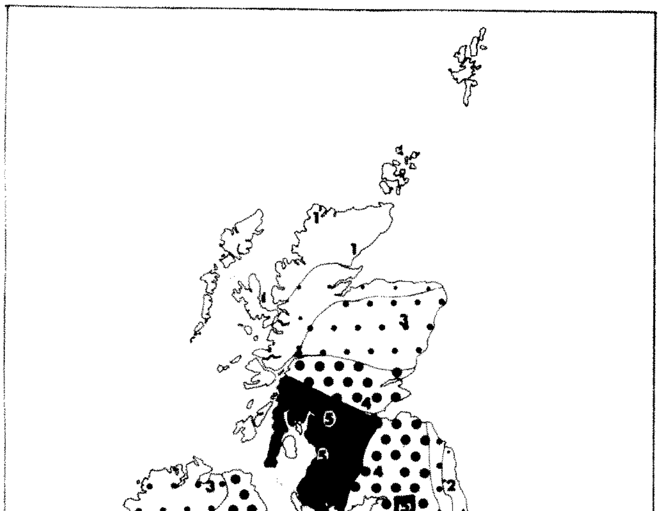

Fig. 2 The distribution of mean values of weekly sunshine hours for the period 13 June–5 September 1977, derived by computer interpolation from Meteorological Office records. The five classes distinguished cover the following ranges of values: 1, 28.9–33.2 h; 2, 33.2–37.5 h; 3, 37.5–41.9 h; 4, 41.9–46.2 h; 5, 46.2–50.5 h.

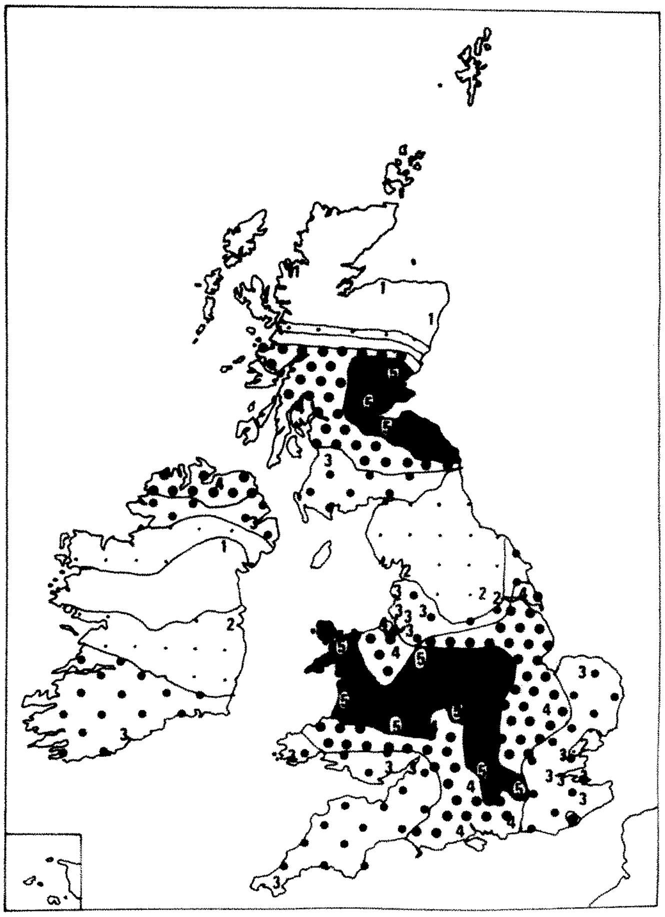

Fig. 3 The distribution of values of k, calculated as described in the text. The five classes cover the following ranges of values: 1, 0.0–0.03; 2, 0.03–0.07; 3, 0.07–0.10; 4, 0.10–0.14; 5, 0.14–0.17.

The levels of sunshine in the summer of 1977 were greatest in the north and west of the country (Fig. 2). As the presence of sunlight is required to drive the reactions leading to ozone formation, its concentrations tend to be high when sunshine levels are high[2,4,5]. Comparing Figs 1 and 2, it can be seen that the highest amounts of leaf injury tended to occur in those areas receiving the greatest amount of sunshine. We found a significant correlation ($r = 0.554$, $P < 0.001$) between the values of mean weekly leaf injury index (LII) and mean weekly (SH) or daily (S) sunshine hours. This relationship could be expressed by the equation $\mathrm{LII} = k\mathrm{SH}$ where $\mathrm{SH} = \Sigma\ (\mathrm{S} - 6.0)$. The value of 6.0 h gives an estimate of the amount of sunshine needed, on average, to produce sufficient ozone to cause leaf injury. The value of k for the fitted relationship was 0.113% h^{-1}. Values of k can also be calculated for individual sites. These values can be used to predict the distribution of leaf injury if the amount of sunshine was uniform over the whole country (Fig. 3). This map shows four bands of alternating high and low leaf injury, lying roughly SW–NE across the country, corresponding to regions of high or low degrees of urbanisation. Meteorological Office records show southwesterly and northeasterly to be the predominant wind directions on sunny days in 1977.

The maps shown here are intended to illustrate general patterns—we do not attach great importance to the precise details of Figs 1 and 3 and concede that the model we have used is a very simple one. It is known that meteorological factors other than sunshine affect the magnitude and distribution of ozone concentrations[1,3–6]. We also concede that the results of one summer must be interpreted with caution; no two summers are the same, especially in Britain. The summer of 1977 was unusually dull, and ozone concentrations were relatively low; stable anticyclonic conditions which are typically associated with very high ozone concentrations[1,3,5–6] were very rare. However, our results do show that, given favourable weather, ozone concentrations sufficient to damage our test plants can occur anywhere in the British Isles, with the possible exception of northern Scotland. Furthermore it would be expected that both the extent and the concentration of ozone would be lower under the climatic conditions of 1977 than in most years.

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^{13}C Content of human collagen as a measure of prehistoric diet in woodland North America

PLANTS with the C_4 dicarboxylic acid pathway of photosynthesis have markedly higher $^{13}C/^{12}C$ ratios than plants with the C_3 or Calvin pathway[1–4]. Cumulative assessments of relative ^{13}C concentration ($\delta^{13}C$) values for C_3 plant foliage yield an average of −26.5‰, relative to the PDB standard whereas C_4 plants average −12.5‰ (ref. 5). These differences in carbon isotope ratios are passed along the food chain to animals and man, so that the nature of the plant food base can be determined through measurements on different body tissues of the consumers. In this study, the time of introduction of maize, a C_4 plant, to the prehistoric inhabitants of several regions in woodland North America, almost exclusively a C_3 plant regime, was determined through isotopic measurements on human bone collagen. The subsequent elevation of maize to the level of a diet staple is also documented.

When plant foods are consumed by animals and man, the $^{13}C/^{12}C$ ratios of the plants are further fractionated in the formation of different body tissues[6,7]. In African browsing ungulates, with diets made up almost exclusively of C_3 foliage, the average $\delta^{13}C$ measurement (relative to PDB) on bone collagen is −21.2‰ (ref. 8). The collagen is apparently enriched by 5.3‰ relative to the food base, but a possible small C_4 plant component in the diet may have added slightly to this enrichment value. Measurements on other body tissues of the same animals yield average $\delta^{13}C$ readings of −21.8‰ for hide and hair, −23.5‰ for flesh and −28.9‰ for fat. It can be calculated from these values that hunter–gatherers who lived in a C_3 environment such as the North American woodlands and who had diets estimated at 20% animal and 80% plant foods had a food web with an average $\delta^{13}C$ value closely approximating that of C_3 plants. The observed addition to their diet of freshwater fish and molluscs would not have changed the situation materially as these organisms also resemble C_3 plants in ^{13}C content. The addition of C_4 plants to the diet will have a profound effect, of course, and the percentage of C_4 plants in the diet should be measurable in the body tissues. This is also true for marine foods in the diet, as marine fauna have an average $\delta^{13}C$ value of about −15‰, with values becoming more negative with decreasing ocean temperatures. These predictions can be seen to hold for a sample of 38 pre-Iron Age Europeans, with a food web based on C_3 plants and a possible marine faunal component, and an average $\delta^{13}C$ value in their bone collagen of −20.4 ± 1.4‰ (refs 9, 10).

Table 1 $\delta^{13}C$ contents of collagen in adult human skeletons from North American woodland sites, relative to PDB standard

Provenance	Sample size & sex	$\delta^{13}C$(‰)	%C_4
Pre-maize			
1. Koster, Illinois; Archaic, ~3000 BC	5 M	−21.7 ± 0.34	0
2. Gibson Mounds, Illinois; Middle Woodland, AD ~200	5 M	−20.9 ± 0.63	0
3. Koster Mounds, Illinois, early Late Woodland, AD ~600	5 M	−20.9 ± 1.3	0
4. Du Pont, Ohio; late Archaic, ~2500 BC	5 F 5 M	−21.7 ± 0.48 −21.9 ± 1.0	
5. Sand Ridge, Ohio; terminal Archaic, ~1500 BC	1 F	−21.4	0
6. Fairchance, W. Virginia; Middle Woodland, AD ~100–200	5 M	−21.1 ± 1.2	0
Average, pre-maize	31	−21 4 ± 0.78	0
Post-maize			
1. Ledders Mounds, Illinois; late Late Woodland, AD ~1000	5 M	−18.1 ± 2.2	~24
2. Schild Cemetery Knoll B, Illinois; Mississipian, AD ~1200	5 M	−14 1 ± 1.1	~52
3. Noble–Wieting, Illinois; early Upper Mississipian	1	−15.1	~45
4. Turpin, Ohio; Upper Mississipian (Ft. Ancient), AD ~1300	6 F 4 M	−12.5 ± 0.55 −11.0 ± 1.4	~64 ~75
Turpin Site average	(10)	(−11.8 ± 1.3)	(69)

To use isotopic measurements as a means of dietary assessment in humans it is necessary to eliminate seasonal factors. Collagen is useful in this respect as its carbon turnover rate is about 10 yr; it is also preserved in archaeological sites. To use collagen, it is also necessary to know accurately the shift in isotopic composition between the plant food base and collagen. We determined this value by measuring the isotopic ratios of collagen from 31 North American skeletons, for an average $\delta^{13}C$ value (relative to PDB) of −21.4 ± 0.78‰ (Table 1). These skeletons derive from hunter–gatherer cultures of Ohio, Illinois, and West Virginia, with archaeologically documented diets of C_3 plants, terrestrial animals and freshwater fish and molluscs[11–16]. A few C_4 species of *Amaranthus*, *Panicum* and *Chenopodia* may have been included in this diet, but apparently in insufficient quantity to have a measurable effect. The consistency of the readings and the wealth of archaeological information on diet make this set of readings the most useful to date for calculating the shift in isotopic composition between the base of the food web ($\delta^{13}C$ = −26.5‰) and human bone collagen ($\delta^{13}C$ = −21.4‰). The collagen enrichment (+5.1‰) was used in the rest of our study to calculate relative contributions of C_3 and C_4 plants in human diets.

C_4 plants include several cultigens of economic importance, for example, sugar cane, maize and sorghum[17]. Their contribution to a diet which is otherwise based on C_3 plants can be

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calculated from isotopic measurements on bone collagen. A diet consisting totally of C_4 plants should yield bone collagen with $\delta^{13}C = -12.6 + 5.1$‰ or -7.5‰. Isotopic measurements between -7.5‰ and -21.4‰ reflect a mixture of C_3 and C_4 diet components. Variation between individuals could change the calculated relative percentage by ±6%, multiple measurements for a site are, therefore, preferred.

In a pilot study of archaeological skeletons from New York[18], a shift away from the C_3-based $\delta^{13}C$ average was observed to accompany the introduction of maize cultivation to the area. This effect is confirmed by measurements on 21 skeletons from sites in Ohio and Illinois (Table 1) where the evidence for maize cultivation is clear. It should be noted that the introduction of maize to this region was also accompanied by the exploitation of imported wild C_4 plants (such as amaranth), which cannot be isotopically distinguished from maize. The contribution of these plants is considered to be minor.

The introduction of maize cultivation in the lower Illinois Valley can be assessed from the isotopic readings. There is no measurable C_4 component in the Archaic (Koster), Middle Woodland (Gibson Mounds) or even the beginning of the Late Woodland period (Koster Mound Group). Occasional maize remains occur in the archaeological record from the Middle Woodland onwards, but in quantities small enough to suggest a trade origin. Exotic artefacts from a wide trade network occur in the same deposits. These sporadic injections of maize in the diet were insufficient to register in the long-term isotopic average of bone collagen. Only in the end of the Late Woodland period (Ledders Mounds, ~AD 1000), when fully agricultural Mississipian cultures were already present in other parts of Illinois, can clear evidence for maize cultivation be seen in a C_4 diet component of ~24%. Thereafter, the importance of the cultigen increased rapidly to ~50% by about AD 1200, among populations represented at Schild Cemetry Knoll B (Mississipian) and Noble–Wieting (early Upper Mississipian).

In Ohio, the measurements now available illustrate the wide gap in isotopic values between hunter–gatherers (Du Pont) and fully agricultural, Upper Mississipian peoples (Turpin). The latter show an average C_4 diet component of ~69%. Separated by sex, the averages are ~64% for six females and ~75% for four males. These results suggest a higher C_3 plant intake among women, perhaps in the course of gathering wild plant foods, and are being checked using a larger population. Further measurements are also underway to pinpoint the 'threshold' of maize cultivation in Ohio, Illinois and other Woodland areas.

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A C4 plant from the Pliocene

THE Kranz syndrome[1,2] is a group of physiological and anatomical features occurring in certain angiosperms; the grasses in particular show increasing efficiency in CO_2 assimilation. Recent research[3,4] suggests an origin of the syndrome in South America and its subsequent dominance in other continents. The Kranz syndrome occurs in all plants fixing CO_2 by phosphoenolpyruvate carboxylase in the mesophyll and subsequent production of Calvin–Benson cycle intermediates in the bundle sheath[5]. The physiological processes in C4 plants are closely related to their specialised leaf anatomy known as Kranz anatomy[6] by which they are distinguished from C3 plants. It is suggested that ancestral non-Kranz C3 grasses originated during the Cretaceous period[4]. We report here fossil evidence indicating that C4 plants occurred at least during the late Tertiary period.

Petrified grasses (Fig. 1*a*) were collected from two localities (Last Chance Canyon and Sperry Hills area) in the Pliocene Ricardo Formation of California. Several specimens from these localities show similarities in their gross morphological features. The culm, present in the majority of specimens, is notched at the distal end and contains a relatively small amount of mechanical tissue. Conversely, at the base of the round culm there are hypodermal bundles imbedded in abundant sclerenchyma. Sheathing leaf bases are imbricate and wrap around the central culm (Fig. 1*d*). Wherever a culm is absent the young leaf at the centre is convoluted. Leaves on specimens from Sperry Wash show marked ridges and furrows. These furrows are probably stomatal locations. Thus, these specimens show Kranz anatomy typical of C4 plants. In addition to a prominent bundle sheath, C4 species are characterised by a low interveinal distance (less than four mesophyll cells between veins) and the presence of radiating parenchyma in the mesophyll[7]. Mesophyll cells in these fossil leaves are occasionally radiating parenchyma. In the absence of radiating parenchyma up to four mesophyll cells are present between the veins (Fig. 1*e*). Certain specimens from the Sperry Hills locality have structural features usually associated with C4 photosynthesis.

Fossil material was subjected to hydrofluoric acid digestion to remove the silicate material. The remainder (mainly cuticle) was then combusted at 800 °C and the CO_2 collected for isotope analysis. The results are presented as $\delta^{13}C‰ = [(R\ \text{sample} - R\ \text{standard})/R\ \text{standard}] \times 1{,}000$, where R = the mass 45 to mass 44 ratio of sample or standard. The standard was PDB (Chicago). C4 plants have Kranz anatomy and $\delta^{13}C$ values of -9 to -16‰ while C3 plants have $\delta^{13}C$ values of -23 to -36‰ and do not exhibit Kranz anatomy. Fossils exhibiting the Kranz anatomy gave a $\delta^{13}C$ value of -13.7‰, indicative of C4 photosynthesis, while non-Kranz fossils gave a value of -24.6‰, similar to C3 plants.

The existence of the C4 photosynthetic pathway is supported by the presence in Ricardo Formation of petrified roots resembling extant panicoid plants. These fossil roots have a central stele surrounded by an aerenchymatous cortex (Fig. 1*c*). Air chambers in the cortex are reticulate as found in *Paspalum commersonii* Lamk. and *P. ciliatifolium* Michx; species of *Paspalum* are known to exhibit C4 photosynthetic pathways[8]. Previous authors have presented evidence for a fruit resembling *Triticum* in the Eocene Deccan Intertrappean beds of India[9] and plants resembling the tribes Stipeae and Paniceae[10] in the Miocene–Pliocene beds in North America.

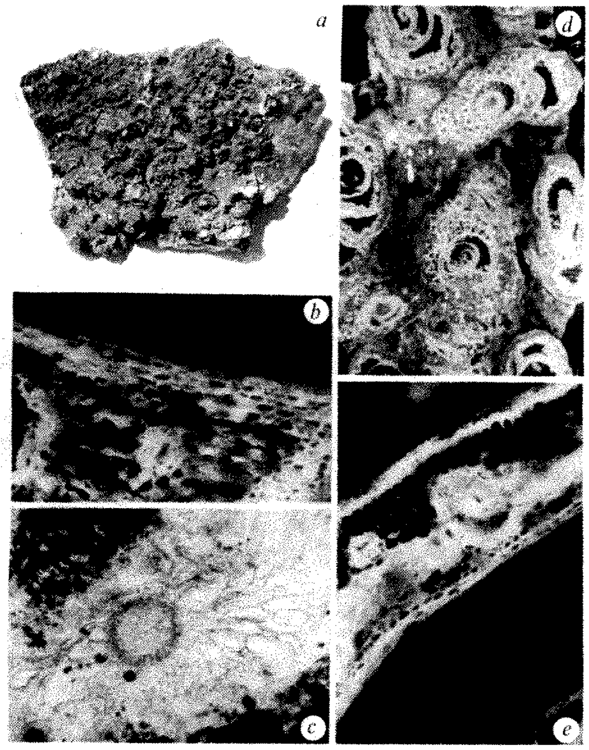

Fig. 1 Petrified grass plants from the Pliocene Ricardo Formation in California. *a*, Distribution of the petrified grass plants in the chert, ×½; *b*, radiating parenchymatous cells of the mesophyll tissue occur between veins, ×100; *c*, petrified grass roots with aerenchymatous cortex resembling the extant genus *Paspalum*, ×105; *d*, sheathing leaf bases wrap around a central culm. In the absence of the culm, leaves at the centre are convolute, ×17; *e*, lamina of the leaf in cross section. Between the veins are about 4 mesophyll cells, ×29.

The presence of petrified grass plants in the Ricardo Formation corroborates the reports on grazing animals in these Pliocene beds[11,12]. Palaeoclimatic determinations are indicative of a plains habitat during Ricardo times[13] with steep slopes bordering plains and relatively less aridity than at present. Such conditions influenced the growth and propagation of grasses as seen in the Chapparal of Mediterranean climates[13].

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Canine tooth size in female primates

AMONG mammals, weapons such as canine teeth or antlers are often more pronounced in males than in females[1–5]. Two reasons are normally proposed for such sexual dimorphism. First, there is more competition among males than among females for access to mates and, in consequence, there is enhanced selection for fighting ability[6–9]. Second, such weapons are important organs of defence for the male, who often assumes the role of defending his mates and offspring from predators[9,10]. In this paper, we discuss a third factor that is involved: selection acting to influence the size of weapons in females.

In primates, sexual dimorphism in canine size seems to be influenced by both inter-male competition for mates and defence against predators by males[9]. Males of polygynous species, where competition for access to mates is intense, have larger canines (relative to their body size and the canine size of female conspecifics) than males of monogamous species. Also, males of terrestrial species, where anti-predator defence is probably important, have larger canines than those of arboreal species. Much of the variance in canine sexual dimorphism remains unexplained, however, probably partly because additional selective pressures affect female canine size. In particular, selection for enhanced female fighting ability (and thus relatively enlarged female canine size) might be expected in monogamous species, where females participate more frequently in territorial encounters and heterosexual aggression than females of polygynous species[11,12]. Of the primates that live in larger groups, female aggression may be more frequent in those species that live in multi-male groups than those living in single-male (harem) groups, for two reasons. First, there is growing evidence[13,14] that female primates may suffer considerable feeding interference from males and it is likely that this will be more intense when there are more males per female in the social unit. (This situation is paralleled in some ungulate species, where females have developed horns or antlers apparently to assist them in feeding competition with males[15–17].) Second, although aggressive inter-female competition over resources may occur in either type of group, females in harems are usually more closely related, and so less competitive with each other, than females living in multi-male groups[7,18,19].

On these grounds, we predicted that females of monogamous species and those living in multi-male groups should have larger canines relative to their body weight than those living in harem groups. Since there is evidence that terrestrial male primates have relatively larger canines than arboreal species[9], we

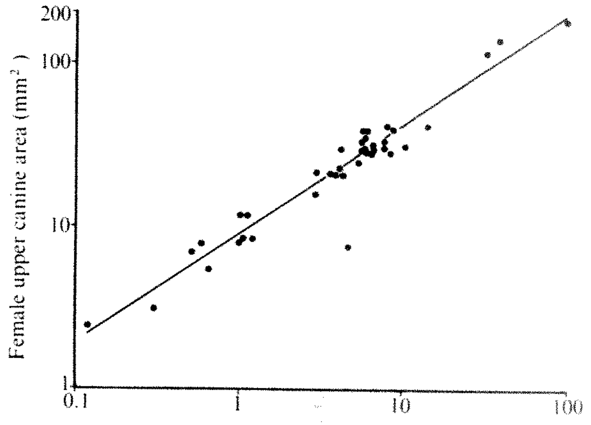

Fig. 1 Female upper canine size regressed on body weight. Both axes are scaled logarithmically.

Table 1 Mean values of relative female canine sizes

	Teeth			
	Upper canine		Lower canine	
	RFCS	n^*	RFCS	n^*
Breeding system				
Harem species	0.818	5	0.861	6
Multi-male species	1.029	15	1.080	14
Monogamous species	1.114	6	1.143	6
Diet				
Frugivores	1.067	25	1.077	24
Folivores	0.926	11	0.945	9
Terrestriality				
Terrestrial species	0.981	8	0.982	8
Arboreal species	1.081	30	1.059	27

* Number of species varies according to availability of data.

examined this relationship in arboreal and terrestrial species separately. Similarly, to allow for any possible effect of diet on female canine size, we have also examined the relationship within folivorous and frugivorous species.

Our measure of canine size is the basal area of the tooth (the product of maximum mesiodistal and maximum buccolingual lengths), which is closely related to canine length ($r = 0.89$, $n = 34$, (ref. 9)). All the dental measurements were extracted from Swindler[20] and are presented elsewhere[9]. Data on body weight, diet and breeding systems are taken from Clutton-Brock and Harvey[21,22]. Female canine size is closely related to body weight (Fig. 1). To remove this effect, we calculated the regression of the natural logarithm of female canine size on the regression of the natural logarithm of non-pregnant body weight for females of 39 species, and measured the deviation in female canine size for each species from the value predicted from female body weight. Our measure of Relative Female Canine Size (RFCS) was the observed canine size, divided by the expected.

As predicted, female primates of harem-forming species have, on average, smaller canines for their body size than do females of other species (Table 1). When the effects of either diet or terrestriality are removed, the differences due to breeding system remain statistically significant for both upper and lower canines (Table 2). There are differences in RFCS between folivorous and frugivorous species, and between arboreal and terrestrial species (Table 1), but when the effect of breeding system is removed, none of these differences is statistically significant (Table 2). In all cases the interaction components are not significant.

Although we have used data from various sources and may therefore have introduced some error[21], our results give statistical support to the hypothesis that selective pressures for female fighting ability are stronger in monogamous species and those living in multi-male groups than in harem-forming primates.

Two possible artefacts may have affected our results. First, if the group sizes of harem-forming species were significantly smaller than those of other species, then this alone might account for the differences between them because intraspecific competition in the former might be relatively reduced by the lower number of animals present. In fact, harem-forming species do not live in smaller breeding or feeding groups than the other primates in our sample[22]. The second possibility is that our results are the product of differences between catarrhine and other primates; catarrhines are noted for their well-developed canines[1,23,24]. It is unlikely, however, that this could explain the relationship between breeding system and RFCS, since all the species in our sample of harem-forming species were catarrhines.

Table 2 Statistical analysis of relative female canine sizes according to breeding system, diet and degree of terrestriality

	Teeth			
	Upper canine		Lower canine	
	$F_{1,22}$ statistic	P	$F_{1,22}$ statistic	P
Terrestriality and breeding system				
Source of variation				
Terrestriality	1.636	n.s.	0.783	n.s.
Breeding system	20.455	<0.001	4.539	<0.05
Interaction	0.097	n.s.	1.222	n.s.
Diet and breeding system				
Source of variation				
Diet	2.200	n.s.	1.385	n.s.
Breeding system	18.091	<0.001	4.596	<0.05
Interaction	0.136	n.s.	2.923	n.s.

Two-way analysis of variance with unequal sample sizes. Although the sample sizes were the same in each analysis, different species were used, according to the availability of data.

n.s., Not significant.

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The distance and nature of the light-trap response of moths

LIGHT TRAPS of various forms have been used to collect and study moths for well over 100 yr, but surprisingly little is known about how they attract moths. There has been some evaluation of the factors influencing the size of light trap catches[1-5] and of the mechanics of the terminal phase of the moth's approach to a light[6], but virtually nothing is understood about the light-trap response itself. Such an understanding is perhaps unnecessary when light traps are used solely to collect specimens, but becomes crucial as soon as they are used for quantitative sampling or survey work[7]. Of particular importance to the interpretation of such work is a knowledge of the distance from which

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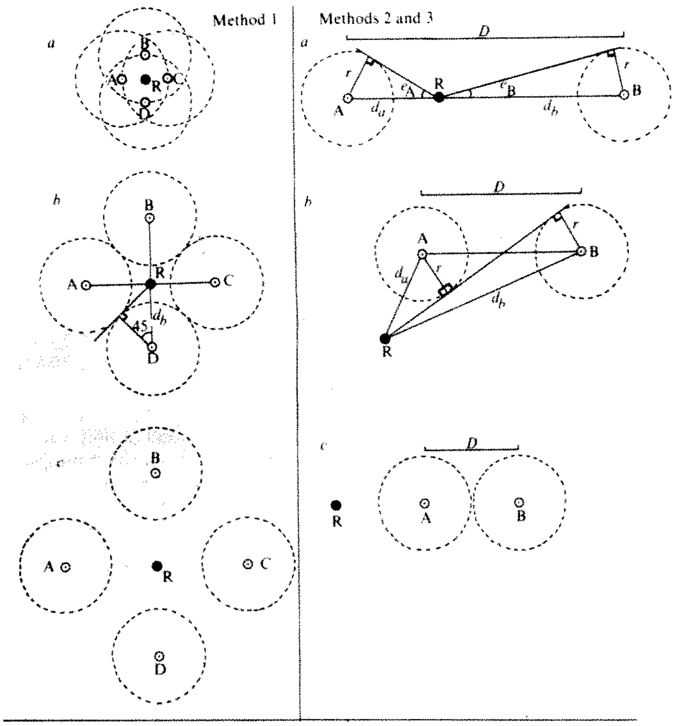

Fig. 1 Three mark-release/recapture methods for estimating the distance of response of moths to a light trap; 1—the increasing circle method. *a*, Four light traps are placed equidistant from a release point (R) at such a distance that the release point is within the response range (dashed circles) of moths to the light traps. The number of marked moths captured by the traps should be relatively high. *b*, As the four traps are moved further and further away from R, there comes a position first at which the edge of the response range withdraws from the release point and second, at which the edges of the response ranges of the four traps cease to overlap but just touch. Even at this position, however, the released moths cannot escape the circle of traps without entering a response range. The proportion of recaptured moths should therefore be the same as for *a*. *c*, Movement of the traps still further from R allows some moths to escape the circle of light traps by flying through the gaps between the response ranges. The proportion of moths recaptured should decrease as the distance of the traps from the release point increases. See Fig. 2(i) for the expected proportion of moths recaptured against distance of the traps from R. The radius of the response range is then given by 0.707 d_b (where 0.707 is the sin of 45° and d is the distance of each trap from the release point at position b). 2—The angle ratio method. a, When R is nearer one trap (A) than another (B), the angle (e_A) subtended to R by the response range of A is greater than the angle (e_B). Assuming that individuals leave R equally in all directions, the ratio (a/b) of marked moths recaptured in A and B should be e_A/e_B. This ratio is a function of r, the radius of the response range ($\sin e_A = r/d_a$; $\sin e_B = r/d_b$). For any given value of e_A/e_B the value of r can be determined by trial and error. 3—The masking method. Starting from the position used for method 2, let the distances d_a and d_b remain constant but let the distance (D) between the two traps decrease (*b*). As D decreases, the release point R swings around trap A. The ratio of marked moths recaptured in A and B should remain constant. This remains true even at the position shown (*b*) where a single line drawn from R is tangential to the response ranges of both A and B. Further decrease in D (*c*) causes the response range of trap B to be increasingly masked by the response range of trap A. The minimum distance possible for D and the maximum masking of B by A is reached at *c*. See Fig. 2*b* for the expected shape of the curve of a/b plotted against D. The point of inflection locates *b* from which, given d_a, d_b and D, r can be calculated:

$$r = \frac{4d_a^2D^2 - (d_b^2 - d_a^2 - D^2)^2}{8d_b^2 + 8d_a^2 - 4D^2}\;^{1/2}$$

Methods 2 and 3 therefore give two independent estimates of r: from the graph, the value (V) of a/b along its horizontal portion gives one estimate (method 2); the point of inflection locates *b* and gives another estimate (method 3).

moths orientate with respect to a light source; it seems intuitively that this distance should be fairly large. We present here the results of three experiments designed to determine the distance of response of free-flying moths to an artificial light source. Our results support Sotthibandhu's claim[4] that the effective range of a 125 W mercury vapour (MV) lamp is about 3 m. They also lead to speculation concerning the behavioural meaning of the light trap response in moths.

There was general agreement that the distance of response to a 125 W MV lamp was of the order of 0.5 km (on a moonless night) (see ref. 8 for review). The first attempt to measure the distance at which individual moths in the field orientate towards such a lamp (positioned 60 cm above ground level) was made by Sotthibandhu[4] who claimed that the distance was of the order of only 3 m. Using flying large yellow underwing moths, *Noctua pronuba*, tethered to apparatus that allowed flight direction to be monitored continuously, he showed that the distance of response to a ground-based light trap varied between 1 and 4 m and was a function of environmental variables such as cloud cover, ambient temperature, and Moon illumination. Further-

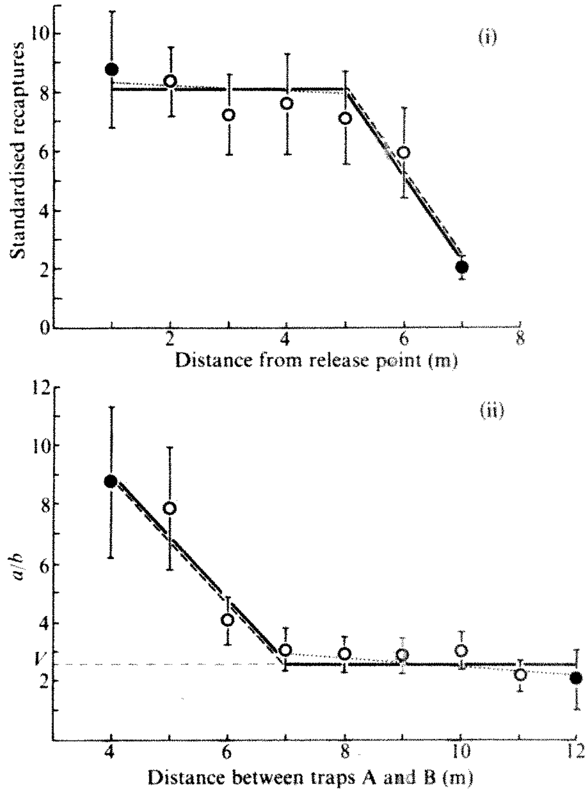

Fig. 2 Analysis of results presented in Tables 1 and 2. Experiments were carried out between 26 June and 26 July 1978 on a lawn at Woodchester Park Field Centre, Glos. Two species of moths, the large yellow underwing, *N. pronuba*, and the heart and dart, *Agrotis exclamationis*, were used. Moths to be marked and released were captured the previous night by the experimental light traps. After marking, without anaesthetisation, with a single dot of cellulose paint on the wing, the moths were stored (in communal keep nets in a cool room) until experimental release at about 22.30 GMT. The moths were released onto the ground within a 10-cm radius of the release point. Dots plus bars indicate means ± s.e.m. ●, means; ○, sliding means for the distance indicated ± 1 m. All regression analyses are for the raw data shown in Tables 1 and 2, not for the means and sliding means. (i) and Table 1 suggest (method 1) that the recaptures start to decrease beyond a release distance of 5 m. Calculated regression lines (dotted and dashed lines) are not significantly different ($P > 0.10$) from the expected (solid) lines. It may reasonably be concluded that the data do not depart significantly from the shape (solid line) predicted by the theory on which the method is based (Fig. 1), given that the response range radius is 3.5 m (that is, 0.707 × 5). (ii) Similarly, the data obtained by the masking experiment (Table 2, Methods 2 and 3) do not depart significantly from the shape predicted (solid line) by the theory on which the method is based (Fig. 1), given that a point of inflection occurs at a value for D of 7 m. The value (V) for a/b over the horizontal part of the curve is 2.09. This is obtained from Table 2 to avoid the effects of Bailey's correction for small numbers. V is calculated for $D \geqslant 7$ by taking the total number of marked moths recaptured in A ($a = 147$) and the total number of marked moths recaptured in B ($b = 42$), correcting for the total number of unmarked moths captured in each (1096 in A, 654 in B), giving $a = 0.134$ and $b = 0.064$, and then dividing a by b. Given that in our experiment $d_a = 4$ m and $d_b = 8$ m, this ratio of 2.09 gives a response range radius of about 2.2 m (Method 2). Given $d_a = 4$ and $d_b = 8$, a point of inflection at $D = 7$ gives a response range radius (r) of 2.7 m (Method 3). Summarising: the three estimates of response range radius are: method 1, $r = 3.5$ m; method 2, $r = 2.2$ m; method 3, $r = 2.7$ m. The three independent estimates of response range given by the three methods are therefore consistent both with each other and with the view that the two moth species studied respond only from a short distance of about 3 m.

Table 1 Effect of varying the distance of release from four equally spaced light traps on the recapture of marked moths (method 1)

			Capture/recapture data for four traps (A, B, C, D)											
			A		B		C		D		A	B	C	D
d_t	date	M	$r+1$	N	$r+1$	N	$r+1$	N	$r+1$	N	R	R	R	R
1	6/7	165	2	8	3	21	3	25	2	29	15 15	8.66	7.27	4 18
2	5/7	143	5	27	3	20	5	47	4	58	12.95	10 49	7.44	4.82
3	7/7	98	2	19	3	111	1	36	1	8	10.74	2.76	2.83	12.76
4	16/7	175	2	27	6	175	2	89	2	8	4.23	1.96	1.28	14.29
5	8/7	177	8	24	14	183	1	21	1	4	18.83	4.32	2.69	14.12
6	22/7	198	6	56	4	43	5	28	2	24	5.41	4.70	9.02	4.21
7	15/7	293	12	118	2	45	2	38	2	49	3 47	1.52	1.80	1.39

d_t, Distance of light traps from release point in metres; M, number of marked moths released; r, number of marked moths recaptured (corrected by Bailey's correction for small samples[16] to give $r+1$); N, number of unmarked moths captured; R, standardised recaptures (that is, number that would have been recaptured if for every night $M = 100$ and $N = 100$), given by $R = [(r+1)100 \times 100]/M.N$. Standardisation was necessary because multiple regression analysis showed the number recaptured to be a significant function ($P < 0.02$) not only of the number released but also of the number of unmarked moths captured.

Table 2 Effect of varying the distance between two traps on the relative proportions of marked moths recaptured in each (method 3)

			Trap A			Trap B			
D	Date	M	r	N	$a = (r+1)/N$	r	N	$b = (r+1)/N$	a/b*
4	30/6	227	49	87	0.575	5	55	0.109	5 27
4	1/7	282	12	50	0.260	2	69	0.043	5.98
4	18/7	174	25	53	0.491	1	61	0.033	14.96
6	9/7	251	38	375	0.104	7	399	0.020	5.19
7	13/7	290	35	457	0.079	3	147	0 027	2.89
8	2/7	415	11	31	0.387	14	118	0.127	3.05
8	3/7	403	38	34	1.147	3	4	1.000	1.15
9	14/7	428	27	282	0.099	5	275	0.022	4.55
10	21/7	65	13	118	0.119	0	22	0 045	2.61
11	24/7	139	6	8	0.875	6	16	0.438	2.00
12	28/6	97	10	156	0.071	3	33	0.121	0 58
12	29/6	279	7	10	0.800	8	39	0.231	3.47

Trap A, 4 m from release point (R), Trap B, 8 m from R; D, distance between trap A and B in metres; M, number of marked moths released; r, number of marked moths recaptured, N, number of unmarked moths captured

* Calculated from a and b before they are rounded to 3 decimal places.

more, for each individual as it was brought nearer to the light, the change-over from celestial orientation[9] to light-trap orientation occurred suddenly at some threshold distance. The implications of such a short threshold response distance of moths to a light trap are far-reaching, particularly with respect to the use of light traps in survey work[2,7], in that, if it is correct, shifts of only a few metres in the position of a light trap could markedly influence the number and species of moths captured. However, it is inevitable that some initial reservations are felt over Sotthibandhu's results because he used tethered moths. There is thus a need for the short-distance nature of the light-trap response to be confirmed using free-flying individuals.

The theory behind our three experimental methods, all of which are based on mark-release/recapture, is presented in Fig. 1. The field procedure is described in the legend to Fig. 2 and the results are presented in Tables 1 and 2. The results are analysed and interpreted in Fig. 2. During the course of this work nearly 5,000 individuals of two species of moth were marked and released. The results (Fig. 2) for all three methods are consistent with the suggestion that moths only respond to a 125 W MV lamp from short distances (about 3 m). Why should moths respond to an artificial light source from such a short distance when it is clear from electroretinographic studies[10] that the light can be perceived from much greater distances?

It has been shown[9] that the angle of orientation (which is individual-specific and which may be anything within the range 0° ± 90° for individuals caught in a light trap) of an individual *N. pronuba* relative to an artificial light source is the same as the angle taken up by that individual relative to the Moon's azimuth. The implication is, therefore, that the light response of moths is a Moon-orientation response, a suggestion first made by von Buddenbrock[11] but which is no longer favoured generally[12,13]. If Buddenbrock was right, the problem remains of why the moths should respond to the Moon at a distance effectively of infinity whereas they respond to an artificial light source at a distance of only 3 m.

Using tethered moths, Sotthibandhu has shown[4] that the response distance to a 125 W MV lamp raised 9 m above ground level is much greater than for the same lamp only 0.6 m above ground level, being of the order of 10–17 m. The angle of elevation of the light source is therefore important, as would be expected if the light-response is a Moon-orientation response. When the light source is near the ground, it is below the height of most flying moths and individuals are often seen to fly overhead, a few metres above ground level, without apparently responding to the light. Others are seen suddenly to dive down to ground level. This behaviour is often a response to bats flying overhead[14] but also frequently occurs in the apparent absence of bats. If the diving response is a response to the light, it could be interpreted as an attempt to restore a Moon substitute, the artificial light, to an acceptable elevation, above the level of the moth's eyes, so that dorsal rather than ventral ommatidia are illuminated[9]. Once a moth is near or on the ground, of course, the elevation of the light can approach that of the real Moon, depending on the distance of the moth from the light. Perhaps the explanation for moths responding to a light 60 cm above ground level, at a distance of only 3 m is that at greater distances, even for low-flying moths, the apparent elevation is not a close enough approximation to that of even a newly risen Moon.

Angle of elevation cannot be the only factor involved in Moon recognition, however, otherwise, when the artificial light is raised 9 m above ground level the distance of response would be expected to be much greater than the 10–17 m observed. Spectral composition, light intensity and the physical size of the light are all factors that could also be important. The light bulbs used by us, which are the same as those used by Sotthibandhu[4], have a horizontal diameter of 7.5 cm and a vertical diameter of 12 cm. As the Moon changes phase its apparent horizontal diameter changes considerably but the vertical diameter is much more constant, subtending to a point on the Earth's surface an angle of 0.518° (diameter = 3,456 km; distance = 382,170 km). The vertical dimension of the light bulb used by us subtends the same angle to a point at a distance of 13.27 m. This is much nearer the distance of response (10–17 m) of a moth near the ground to an elevated (9 m above ground level) light source than is the distance (35–519 m, depending on Moon phase, latitude, and so on[8]) at which the light source has the same level of illumination as the Moon.

We suggest, therefore, that the light-trap response of moths results when the moth ceases to recognise that the artificial light is not the Moon, and that the most important features used in this recognition are first, the elevation, and second, the vertical diameter of the light source. Level of illumination and spectral composition may also be involved[5,8] but are suggested here to be far less important.

On the interpretation of the light-trap response offered here, light traps should catch only those individuals that are in the physiological state in which they would make use of Moon orientation. Most often these will be individuals that are in the process of, or just initiating or terminating, migration. This could explain why light traps do not capture all species of moths in the vicinity[2] (non-migrants not showing the light-trap response) and why males of most species predominate in light-trap catches, males probably being more migratory than females[15]. It could also explain why a better correlation is obtained between light trap catch and volume of migration as determined by radar[3] than between light-trap catch and suction-trap catch[2].

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Male mating experience and competitive courtship success in *Drosophila melanogaster*

MULTIPLE copulations by *Drosophila melanogaster* males reduce their fertility[1], even though these males will continue to court and mate after four or five successive matings. This sterility involves depletion of the accessory glands and not of sperm supply[2] and is only temporary, for when these males are mated 2 or 3 h after the onset of sterility, they once again transfer sperm. A female *Drosophila* would maximise her reproductive fitness by mating with a male which transfers the most sperm; however, virgin females will copulate with temporarily sterile males when placed in a single-pair situation. Although female *Drosophila* are capable of discriminating mates on the basis of genotype differences[3], it is not known whether they will selectively copulate with fertile males in competition with temporarily sterile males of the same strain. In the experiments reported here, we have examined the relative mating success of experienced males with temporarily reduced fertility and virgin males from the Canton-S strain of *D. melanogaster.* The data show that when a virgin female is placed with a virgin and an experienced male, she tends to mate with the virgin male. Examination of some possible factors for increased virgin male success, such as time until initiation of courtship, amount of time spent courting, and overall mating speed, showed that for the courtship parameters measured, virgin and experienced males are equal.

Flies were raised on standard cornmeal–molasses–agar medium at 24 ± 1 °C. Virgin flies were separated by ether anaesthetisation and stored until 3 d old. Virgin females were then placed individually into 8-dram shell vials containing one experienced male and one virgin male. Males were distinguished by wing clipping[3], which was done at the time of collection to avoid the need for a second anaesthetisation. Clipping was alternated between groups to avoid any possible effects on the results. Small clips have not been found to alter mating in the present study (see below) or in previous studies[4]. All subsequent handling of flies was done by aspiration.

Table 1 Number of offspring produced by males mating three successive times

Mating	Mean no. of offspring per mating per male*	No. of males
1	288.0 ± 17.4	33
2	296.1 ± 28.2	31
3*a*	194.7 ± 23.6	30
3*b*	206.0 ± 21.0	29
3*c*	303.2 ± 18.8	35

a, Males mated immediately after the second mating; *b*, males rested 1 h after the second mating; *c*, males rested 24 h after the second mating.

* A Duncan multiple range test places these data in two statistically significantly different subsets ($P < 0.01$) where offspring number from mating 1, 2 and 3*c* are in one subset, and offspring numbers from matings 3*a* and 3*b* are in another subset.

Three series of experiments were carried out. In series 1, 'experienced' males were obtained by placing virgin males in a shell vial containing one virgin female. Immediately after copulation, the male was placed with another virgin female. During the initial phase of the experiments, 97% of the original males had mated the first and second times by the 1-h cut-off period. After the second mating, each experienced male was placed with a virgin male in a clean shell vial, and a virgin female was introduced. Courtship latency, duration of courtship, successful male and duration of copulation were recorded. Vials showing no mating in 1 h were discarded. In series 2, males were handled the same way except that they were stored alone for 1 h between the second and third matings to eliminate any possible effects of postcopulatory preening or inactivity. In series 3, males were stored for 24 h before the third mating and tested with virgin males of the same age.

The number of offspring produced by males mating three times was determined by saving the females from each of the three matings and counting their progeny (Table 1). Fertility is greatly reduced at the third mating when this occurs within minutes of the second mating or when males are rested for an hour. When males are rested for 24 h after the second mating,

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their fertility rises to levels seen in once-mated males. The total number of offspring produced by virgin males is slightly less than reported elsewhere[2]. However, the present study used a different wild-type strain.

Table 2 shows the relative mating success of experienced males. The data for the nine replications ($n = 15-20$ per replication) in series 1 were pooled following testing for homogeneity[5]. The numbers shown for series 2 are pooled values for nine replications, and for series 3 eight replications. In series 1 and 2, virgin males had a distinct mating advantage. In series 3, no advantage existed.

Table 2 Relative mating success of virgin and experienced *D. melanogaster* males

		Male chosen				
Series	% Mating	Virgin	Experienced	Total	X^2	P
1	95	115	65	180	13.88	<0.01
2	96	104	74	178	5.02	<0.05
3	96	84	91	175	0.28	NS

Overall clipping $x^2 = 0.965$. NS, not significant.

It has been reported that in *Drosophila*, the females 'decide' if and with which male a mating will take place[3,6]. Obviously, these 'decisions' depend on various sensory cues from the males. It is known that males which spend less time courting have a reduced mating success[7]. However, the disadvantage of the males with reduced fertility in the present study could not be explained by any reduction in observable courtship activity. Experienced males from series 1 and 2, whether successful or not, equalled virgin males in (1) being first to begin courtship, and (2) the proportion of time spent not courting. The average courtship times in series 1 and 2 were 6.01 ± 0.29 min for successful virgin males and 5.97 ± 0.33 min for successful experienced males ($t = 0.024$, NS). The duration of copulation was 20.61 ± 0.91 min and 21 ± 1.06 min for virgin and experienced males, respectively ($t = 0.76$, NS).

Although the virgin and experienced males of series 1 and 2 were of the same age and strain, they are in some way perceived as differing from each other by females. As the standard tests of courtship which would reflect differences in male vigour cannot explain the differences in success of the two types of males, the possibility that they are characterised by different olfactory stimuli[8], or patterns of organisation of courtship elements[9] should be investigated. Some stimuli from the males must somehow be influencing the mate selection process of the females. Any mechanism which results in females discriminating the level of fertility of prospective mates would certainly have a selective advantage. Other species should be studied to determine how widespread this phenomenon is.

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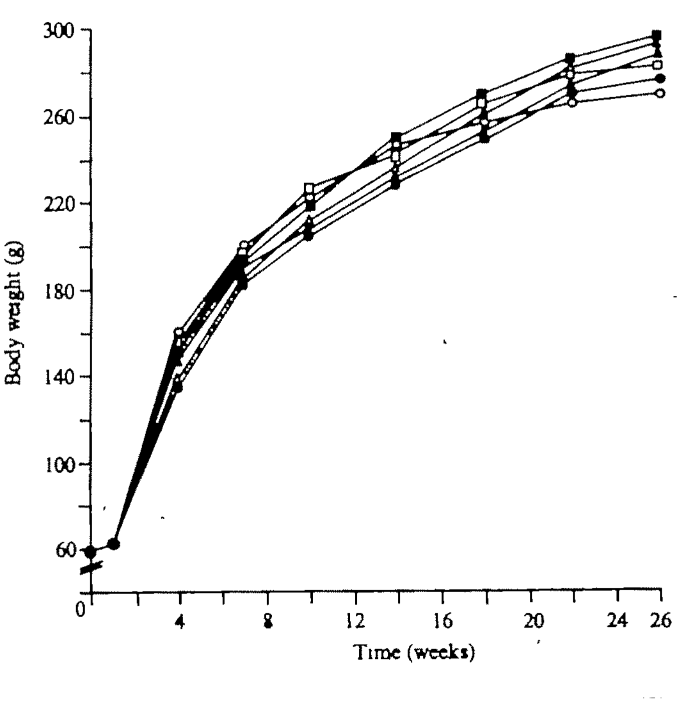
Co-carcinogenic effects of dietary cholesterol in experimental colon cancer

COLORECTAL CANCER is now the most common internal malignancy in the US (ref. 1), but its aetiology is unknown. Western dietary factors are likely to be implicated[2,3], and high fat diets correlate well with the geographical incidence of colon cancer[4–6]. Patients with colon cancer have high levels of faecal bile acids and cholesterol[7] as do patients with diseases known to be associated with colon cancer, for example, familial polyposis[8] and ulcerative colitis[9], and also other high-risk populations[10]. It is thought that faecal bile acid and cholesterol metabolites may act as promoters, co-carcinogens or carcinogens in large bowel tumorigenesis[11–13]. As cholesterol is the obligatory precursor of the bile acids[14], we have tested the ability of dietary cholesterol to promote the induction of colon cancer by dimethylhydrazine (DMH) in the rat. We report here evidence from an animal study that cholesterol is a potent dietary co-carcinogen.

The experimental method and diets used are detailed in Table 1. The effects of a single dietary variable, cholesterol, was investigated on the induction of colon cancer in two identical groups of rats, both fed on Vivonex, a cholesterol-free, chemically defined liquid diet. One group (V) received unmodified Vivonex, and the other group (VC) received Vivonex with added cholesterol. The third dietary group (S) was included solely for comparison as a carcinogen-injected control group and was fed a standard formula solid diet. By design, all three diets were fibre-free and provided equivalent caloric intakes.

The results were analysed after 56 weeks' observation. During the first 6-month period, animals on all dietary regimens maintained comparable weight gains (Fig. 1). Any observed differences between groups are therefore unlikely to be attributable to the effects of caloric restriction. Comparisons beyond 6 months are inaccurate, because of variations between sick animals in relation to appetite and weight loss due to the induced colon cancers. At 56 weeks, all control animals were alive and well, except for a single rat fed on Vivonex plus cholesterol

Fig. 1 Growth curves showing weight gain of rats on various dietary regimens. Each point represents a mean weight of the 10 rats in each group. Standard deviations have been omitted for clarity. Statistical comparison shows no significant differences between groups at 26 weeks ($F = 0.28$, d.f. = 5,54, $P > 0.10$). Groups: ○, S, ●, V control, △, V; ▲, VC; □, VC control; ■, S control.



which died of pneumonia at 42 weeks with a tumour-free colon. All the remaining deaths occurred in the carcinogen-treated rats and (with two exceptions cited in Table 2) all were attributable to histologically confirmed adenocarcinoma of the colon, or its complications. All animals either died spontaneously (44.4%) or fulfilled one of the recently published[17] objective criteria for killing (55.6%) (ref. 17).

Examination of the results (see Figs 2, 3 and Table 2) shows that DMH-treated animals fed on the cholesterol-free diet Vivonex showed a highly significant increase in both time to tumour presentation ($P < 0.001$) and survival ($P = 0.001$) when compared to carcinogen-injected controls fed on a standard diet. This suggests that Vivonex protected against DMH carcinogenesis. Comparison between the two groups of Vivonex-fed animals (groups V and VC) shows that the addition of cholesterol to Vivonex significantly reduced both the time to tumour presentation ($P < 0.001$) and survival ($P = 0.008$) of group VC, as well as increasing the incidence of metastases ($P = 0.028$). The latter finding was particularly striking (see Table 2) as 67% of group VC animals had abdominal carcinomatosis at necropsy. Distant metastases are uncommon in this model[20] and in our experience the incidence rarely exceeds 20%. This suggests that the addition of cholesterol to Vivonex not only abolished the protective effect of Vivonex, producing similar tumour indices in group VC to those of group S, but also enhanced the spread of the induced colonic cancer. These findings are attributable only to the single experimental variable, the provision of cholesterol in the diet.

Table 1 Dietary intake and experimental method

Dietary component	Calculated range of daily intake in each dietary group (mg per adult rat per day) Vivonex[*] (V)	Vivonex plus cholesterol[†] (VC)	Standard[‡] (S)
Cholesterol	0	6–9[§]	3–5
Saturated fat	0	0	105–140
Unsaturated fat	65–98	65–98	363–484
Kilocalories per kg	750	750	3580
Consumption per adult rat per day	60–90 ml	60–90 ml	15–20 g
Kilocaloric intake per adult rat per day	45–68	45–68	54–72

Sixty weanling inbred female Wistar rats (A. Tuck) weighing 60–70 g were weaned on to a standard diet (41B), for one week to establish normal colonic function and bacterial colonisation. Thereafter, they were randomly allocated in groups of 20 to one of the three dietary regimens. After a further two weeks on their respective diets, half the animals ($n = 10$) in each group were injected with carcinogen DMH dihydrochloride (97%) (Aldrich) at a dosage of 40 mg per kg body wt per week subcutaneously for 13 weeks and prepared according to the method of Filipe[16]. The other half received equivalent volumes of saline preservative to act as controls. They remained on these diets for the rest of their lifetime. Animals were housed in temperature-controlled quarters in subgroups of five in suspended cages which had open-mesh, wire floors designed to prevent coprophagia. They were weighed weekly and inspected daily for signs of illness or colonic disease (for example, rectal bleeding with positive faecal occult blood test, tumour prolapse per rectum, diarrhoea, ascites or colonic obstruction with abdominal distension). When moribund, animals were isolated to prevent cannibalisation, and either died spontaneously or were painlessly killed when they fulfilled objective criteria for death[17]. Complete necropsies, including the selection of specimens for histological examination, were performed on all animals. Four tumour indices (see Table 2 for definitions) were measured in each animal during this *in vivo* study. The time to tumour presentation and survival were analysed statistically by the log rank method of Peto *et al.*[18]

[*] Vivonex was prepared each day as follows: 10 sachets (each providing 300 kcal) were dissolved in 3 l of fresh water, mechanically stirred in a 5 l conical glass flask until fully dissolved, and made up to a final volume of 4 l. It was then divided into 2 equal volumes, to one of which 200 mg cholesterol was added, and both volumes were stirred for 1 h. The liquid diets were then dispensed into standard 540-ml glass feeding bottles and offered to the animals at the same time each day.

[†] 99.9% free of solid impurities (BDH Chemicals).

[‡] Formula Diet 41B, in pellet form (BP Nutrition). This diet was specially prepared to exclude all crude and dietary fibre.

[§] This dosage was selected to approximate, on a body weight basis, the daily cholesterol intake of a 70 kg man breakfasting on two fried eggs and two rashers of bacon, that is, 1,000 mg per day[15].

Cholesterol has not yet been shown to be a colon carcinogen, although there is evidence[21], recently disputed[22], that it is sarcomagenic in mice. However, the control animals receiving added dietary cholesterol in our experiment are alive and clinically tumour-free, and the observed effects cannot therefore be ascribed to cholesterol *per se*. As the augmentation of DMH colon tumour induction by cholesterol is within Berenblum's definition of 'co-carcinogenic action'[23], the effects can be attributed to cholesterol acting as a dietary co-carcinogen. We conclude, therefore, that dietary cholesterol facilitates the development, growth and spread of this experimental colon cancer.

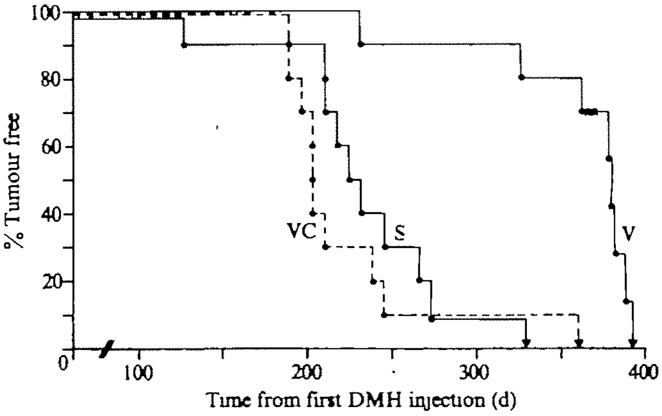


Fig. 2 Life table of proportion of tumour-free animals as a function of time after first injection of DMH. ●, Tumour presentations, ×, deaths without presentation. Statistical analysis of time to tumour presentation[18]:

Group	Observed nos presenting (O)	Extent of exposure (E)	Ratio (O/E)
V	8	17.70	0.45
VC	10	4.21	2.38
S	10	6.09	1.64

V against VC, $\chi^2 = 14.16$, 1 d.f., $P < 0.001$; VC against S, $\chi^2 = 0.27$, 1 d.f., $P > 0.10$; V against S, $\chi^2 = 13.59$, 1 d.f., $P < 0.001$.

Evidence from other animal studies has shown that high fat diets[12,24–26] and bile salts[27,28] promote the effects of several large-bowel carcinogens, and that animals on such high fat diets excrete more faecal neutral sterols and bile acids than do controls[12]. However, there is disagreement as to the type of dietary fat responsible for the promotion. Studies comparing the relative role of saturated with unsaturated fats did not detect differences in colon tumour incidence[25,29], but a recent report associates an increased tumour yield with a diet high in unsaturated fat and cholesterol[26]. Such discrepancies are due, in part, to the fact that many experimental diets contain mixtures of fats and variable amounts of non-fat components such as fibre which may exert independent effects[25]. The use of Vivonex obviates this problem, as it contains neither cholesterol, saturated fats nor dietary fibre and therefore allows carefully controlled dietary alterations. It has been used before in this model by Castleden[30], but the experiment was terminated at 24 weeks; the Vivonex diet used was not fibre-free, as the rats had been eating sawdust as well.

Two salient questions are raised by our results. The first relates to the mechanism whereby Vivonex feeding protects against DMH colon carcinogenesis, and the second to the mechanism whereby cholesterol overcomes this protective effect and facilitates tumour development. The effects of both Vivonex and cholesterol on DMH metabolism are unknown. Vivonex has been shown in humans to affect faecal bulk, transit time and flora, as well as reducing the levels of faecal bile acids and cholesterol metabolites[31], and some or all of these factors may be involved. However, as our evidence demonstrates that the addition of cholesterol to Vivonex overcomes the protective effect, the simplest explanation is that Vivonex protects because it lacks cholesterol. The protection is clearly incomplete, as Vivonex-fed rats do eventually develop colon cancers. This could be attributed to endogenous synthesis of cholesterol (or its metabolites), or the presence of a small amount of polyunsaturated fat in Vivonex, or a combination of the two. However, it is possible that the induced colon cancers could progress spontaneously in the complete absence of cholesterol, both exogenous and endogenous, due either to random biological accident or to another qualitatively different cause.

The relevance of animal data to the human situation is debatable, but the concepts are useful, particularly when seen against the general framework of multi-stage tumour models. Modern theorists[32,33] envisage a multi-stage transition from a normal to a cancerous state. In general, the early stages seem to be initiated by mutational events, with subsequent stages proceeding either randomly, by biochemical accident, or at a rate determined by various extrinsic agents. In this context, a parallel may be drawn between animal and human colon carcinogenesis: in the animal model, DMH (a mutagen) presumably affects one or more early stages, and dietary components such as fat and cholesterol one or more later stages. Similarly, whilst the early (presumably mutational) stage in the genesis of human colon cancer is unknown, the disease does seem to involve multi-stage processes[34], and an interpretation of the data implicating dietary fat and cholesterol in man would be that these dietary components are analogous rate-determining extrinsic agents affecting the later stages.

Although it is possible, in both animals and man, that the different stages could proceed in the absence of dietary fat or cholesterol, the experimental and epidemiological evidence is consistent with the interpretation that these dietary components are associated with an apparent acceleration of the processes involved. Our observations corroborate this concept, and specifically suggest that dietary cholesterol is a factor determining the rate of development of experimental colon cancer. If this applies to man, as the metabolic evidence would suggest, then human colon cancer may be a preventable disease.

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Table 2 Effects of dietary cholesterol on DMH-induced colon cancer in rats, analyses of results 56 weeks after first carcinogen injection

Dietary group ($n = 10$)	Rat code	Time to tumour presentation* (d)	Survival† (d)	No. of colonic tumours per rat‡	Distant abdominal metastases§	
Vivonex (V) (Cholesterol-free)	V1	231	278	1	No	
	V3	326	326	2	No	
	V7	>362	362	0	No	0/5
	V5	362	362	7	No	
	V8	>369	369	1	No	
	V10	378	Alive at 400 days			
	V2	380				
	V9	382				
	V6	388				
	V4	392				
Vivonex plus cholesterol (VC)	VC4	189	236	2	Yes	
	VC3	189	238	4	No	
	VC7	196	238	5	Yes	
	VC5	203	263	8	Yes	
	VC1	203	286	10	No	6/9
	VC2	203	298	11	Yes	
	VC9	210	328	5	Yes	
	VC6	238	340	4	Yes	
	VC10	245	357	6	No	
	VC8	360	Alive at 400 days	–	–	
Standard (S)	S8	126	132	1	No	
	S1	210	217	3	No	
	S5	210	218	7	No	
	S4	217	224	4	No	
	S10	224	286	4	Yes	
	S6	231	306	7	No	2/10
	S2	245	319	2	Yes	
	S7	266	329	3	No	
	S3	273	342	3	No	
	S9	329	369	9	No	
All controls ($n = 30$)	VC Control 2	–	297	–	No	

* Defined as time in days from first carcinogen injection to the first sign of colonic disease. Statistical analysis, see legend to Fig. 2.

† All deaths attributable to colon cancer, except single control death. Animals V7 and V8 were killed for humane reasons after they had developed large primary malignant renal tumours. Neither had colonic carcinoma at histology. Statistical analysis, see legend to Fig. 3.

‡ Macroscopic tumours counted at necropsy. At least one tumour per animal was histologically confirmed as a primary adenocarcinoma of the colon. Statistical analysis, no significant differences between groups using Mann–Whitney test[19].

§ Distant abdominal metastases (to regional nodes, omentum, peritoneum and liver), confirmed histologically. Statistical analysis, the incidence of metastases in group VC was significantly greater than that in group V ($P = 0.028$) using the one-tailed Fisher exact test[19].

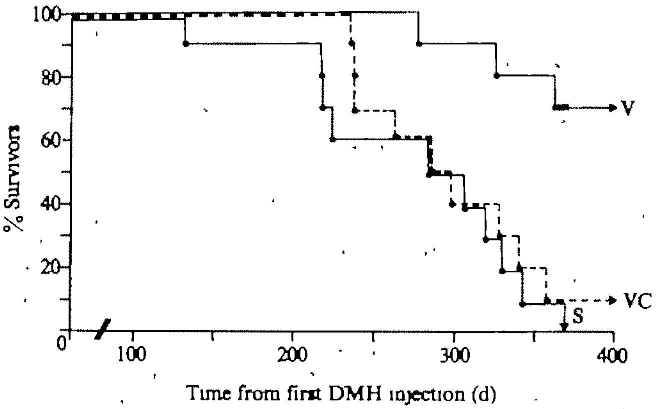

Fig. 3 Life table of proportion of survivors of death from colon cancer as a function of time after first injection of DMH. ●, Deaths from colon cancer; ×, deaths from other causes. Statistical analysis of survival[18]:

Group	Observed survival (O)	Expected survival (E)	Ratio (O/E)
V	3	10 98	0.27
VC	9	6.01	1.50
S	10	5.01	2.00

V against VC, $\chi^2 = 6.96$, 1 d f , $P = 0.008$; VC against S, $\chi^2 = 0$ 16, 1 d.f., $P > 0.10$; V against S, $\chi^2 = 10.33$, 1 d.f., $P = 0.001$.

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Protease inhibitors suppress radiation-induced malignant transformation *in vitro*

LITTLE is known about the mechanisms of carcinogenesis. The fact that most carcinogens are mutagenic has led to speculation that the primary step in cancer induction may be mutational[1,2]; there is evidence from both *in vivo*[2] and *in vitro*[3] studies that a strong correlation exists between the mutagenicity and carcinogenicity of an agent. Mutagenic and carcinogenic agents, both physical and chemical, also produce similar kinds of DNA damage and repair[4]. Radiation-induced mutagenesis in some bacterial cells requires an error-prone DNA repair system[5,6], and there is now some evidence that error-prone DNA repair may be involved in the malignant transformation of cells by radiation[7,8]. Protease inhibitors have been shown to suppress specifically both error-prone repair and mutagenesis in bacterial cells[9,10], as well as to inhibit carcinogenesis *in vivo*[11,12]. We report here that the protease inhibitors antipain and leupeptin will suppress radiation-induced transformation *in vitro* as well as inhibit two-stage transformation *in vitro* using radiation and the promoting agent, 12-O-tetradecanoyl-phorbol-13-acetate (TPA).

We used a C3H mouse-embryo-derived cell line (10T$\frac{1}{2}$ clone 8) isolated and characterised by Reznikoff *et al.*[13,14], and adapted for studies of radiation transformation[7,15,16]. In some experiments, a BALB/3T3 mouse line designated A31-11 was used. Concentrations of the protease inhibitors used were nontoxic, either with or without radiation exposure, and have been shown not to affect growth, RNA or protein synthesis in bacterial[9] or mammalian cells[18–20].

The influence of antipain and leupeptin on X-ray induced transformation is shown in Table 1. In these experiments, the inhibitors were present for the entire post-irradiation expression period. As can be seen, antipain not only suppressed X-ray-induced transformation but also completely abolished transformation enhanced by TPA. Similarly, leupeptin suppressed radiation transformation enhanced by TPA, although this protease inhibitor did not seem to be as effective as antipain. To determine whether these protease inhibitors were effective in suppressing radiation transformation when present for only a short post-irradiation interval, experiments were carried out with antipain present only during the 5-d or 1-d period following radiation exposure. Antipain at 1 mM (600 μg ml^{-1}) reduced transformation from 2.0×10^{-3} to $<3.2\times10^{-4}$ when present for 5 d and to 5.5×10^{-4} when present for only 1 d following 600 rads.

A preliminary experiment was carried out with A31-11 mouse BALB/3T3 cells to determine whether the suppressive effect of antipain was cell line-specific. These results are shown in Table 2. Again, incubation with antipain (50 μg ml^{-1}) throughout the expression period completely suppressed transformation induced by 100 or 400 rad of X rays plus TPA exposure.

Endogenous proteases have been implicated previously in the mechanism of tumour promotion[21], and protease inhibitors have been shown to suppress two-stage carcinogenesis *in*

Table 1 Effects of 6 weeks post-irradiation exposure to antipain and leupeptin on X-ray transformation of 10T$\frac{1}{2}$ cells with and without promotion by TPA

Treatment	Expt. no.	PE %	Total no. cells at risk	Total no. transformed foci*	Transformation frequency per viable cell ($\times10^{-4}$)*
Controls (no treatment)	1	32.5	3,120	0	<3 2
	2	29.3	4,395	0	<2.3
Antipain alone	1	32.2	3,088	0	<3.2
	2	32.3	4,845	0	<2.1
600 rads	1	3.0	2,160	5	23.1
	2	2.4	7,200	11	15.3
600 rads + antipain	1	3.4	3,264	1	3.1
	2	2.7	3,120	0	<3 2
600 rads + leupeptin	1	3.8	3,192	1	3.1
	2	2.9	3,780	2	5.2
400 rads	1	6.3	3,402	3	8.8
	2	4.9	7,200	4	5.5
400 rads + TPA	1	7.0	2,940	9	30.6
	2	5.7	7,140	22	30.8
400 rads + TPA + antipain	1	7.4	1,332	0	<7.5
	2	7.7	4,620	0	<2.2
400 rads + TPA + leupeptin	1	10.6	5,088	2	3.9

Details of experimental techniques for radiation transformation experiments using 10T$\frac{1}{2}$ cells have been described elsewhere[7,15,16]. Stock cultures were maintained in 60 mm Petri dishes and were passed by subculturing at a 1 : 20 dilution every 7 d. The cells used were in passages 9–14. They were grown in a humidified 5% CO_2 atmosphere at 37 °C in Eagle's basal medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics. Cells were irradiated 24 h after seeding, and incubated in fresh medium containing TPA and/or protease inhibitors immediately afterwards. Antipain and leupeptin added at 50 μg ml^{-1}; TPA at 0.1 μg ml^{-1}.

* Types 2 and 3 foci were scored as transformants, as described by Terzaghi and J.B.L.[15].

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Table 2 Effect of antipain on oncogenic transformation induced in 3T3 cells by X rays plus TPA

Treatment	Total no. cells at risk	Total no. transformed foci	Transformation frequency ($\times 10^{-4}$)
400 rads	60,500	66	10.9
400 rads + antipain	43,450	14	3.2
400 rads + TPA	52,800	91	17.2
400 rads + TPA + antipain	50,050	0	<0.2
100 rads + TPA	34,400	29	8.4
100 rads + TPA + antipain	33,500	0	<0.3

Stock cultures were passaged as for 10T½ cells. Cells were seeded 24 h before irradiation at densities which yielded about 4,000 viable cells per 100 mm Petri dish. They were grown in Eagle's minimal essential medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics. Transformed foci were scored at 4 weeks[17]. Antipain was added at 50 μg ml^{-1} (0.08 mM) and TPA at 0.1 μg ml^{-1}.

vivo[11,12]. It is possible, therefore, that the suppression of radiation transformation by these protease inhibitors is due entirely to an effect on the promotion stage of transformation (or carcinogenesis). This hypothesis is supported by the observation that almost complete suppression occurred in the presence of TPA. However, the fact that antipain was also effective in inhibiting transformation at early stages (within the first 1 or 5 d of irradiation) argues against this hypothesis, and suggests that an effect on DNA repair processes might be important in its action. TPA is ineffective in enhancing X-ray transformation unless it is present for at least 14 d or the entire proliferative phase of expression after irradiation (unpublished data).

Mutagenesis in some bacterial cells requires an error-prone DNA repair ('SOS repair') function[5,6]. It has been postulated that the induction of mutations in mammalian cells may also be associated with an error-prone DNA repair process[22,23]. There is some evidence that DNA repair mechanisms operating within the first few hours of radiation exposure may be involved in the induction of malignant transformation by X irradiation in 10T½ cells[7,8]. The theory that proteases have the ability to cleave suppressors of error-prone repair in bacteria and thus induce this type of repair[5,6,9] suggests that protease inhibitors might inhibit malignant transformation *in vitro* by inhibiting error-prone repair. Thus, it is possible that the action of protease inhibitors on a DNA repair process may be a mechanism for our observed suppression of radiation transformation. Further investigation of this hypothesis will require a careful examination of the post-irradiation intervals during which antipain has its greatest effect on transformation, as well as its effect on specific molecular repair processes during these times.

In any event, the present results offer *in vitro* confirmation for the suppressive effect of protease inhibitors on carcinogenesis *in vivo*. They suggest that a further examination of the mechanisms of action of these agents may offer valuable insights into mechanisms of carcinogenesis.

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Immunological properties of the surface of parasitic nematodes

THE surface of parasites seems to be especially important in the intricate relationship which is known to exist between these organisms and their hosts. This is particularly clear in the case of African trypanosomes, which are able to vary the antigenic glycoproteins of their surface sequentially during infection[1]. It is also known that the surface of schistosomes changes during infection in parallel with alterations in the susceptibility of this trematode to the action of immunity[2]. By comparison with these and other parasites, little is known of the properties of the surface of nematodes nor of its role in the host–parasite interaction. Both free-living and parasitic nematodes are bounded by a non-cellular cuticular layer which is probably living because it may contain, for example, enzymes and haemoglobin[3]. The cuticle, which is shed and replaced as the nematodes pass through the various moults in their life cycle, consists of fibrous collagen-like proteins, thought unlikely by some authors to be highly immunogenic in the host[4]. However, we now report that

Table 1 Viability of *T. spiralis* newborn larvae cultured *in vitro* for 30 h with peritoneal cells and immune serum

Source of serum	Source of peritoneal cells	% Parasites dead*
Normal rat	Normal rats	2.2
	Infected rats	0.5
Normal rat, heat inactivated	Normal rats	5.8
	Infected rats	2.1
Immune rat	Normal rats	93.7
	Infected rats	98.6
Immune rat, heat inactivated	Normal rats	66.6
	Infected rats	36.1

Immune serum was taken at either the 18th or 24th day after a primary infection with 4,000 infective larvae. Inactivation was achieved by maintaining serum at 56 °C for 1 h. Cells from infected rats were taken from the peritoneal cavity of rats 24 d after a primary infection and prepared as described in Fig. 1. This suspension contained 35–45% eosinophils, 5% mast cells and 50–60% mononuclear cells.

* 150 Parasites per replicate were observed with at least four replicates in each group. The parasites were considered to be dead when they were completely immobilised and had visible signs of deterioration in their internal organs.

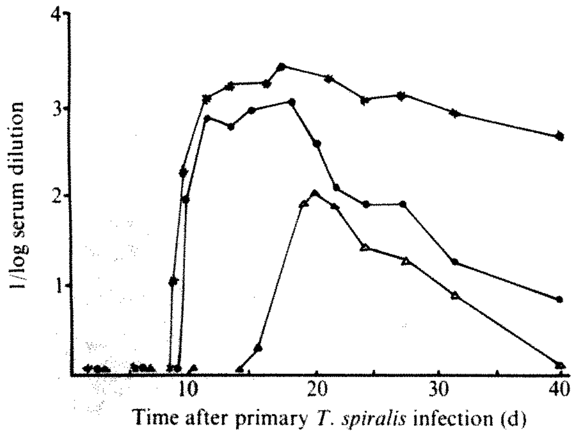

Fig. 1 Serum studies in August rats infected with 4,000 infective larvae of *T. spiralis*. The time after infection to appearance of antibodies mediating *in vitro* adherence of a population of peritoneal cells to newborn larvae (△), infective larvae (*) and adult parasites (●). Sera obtained at various times after infection were inactivated by heating at 56 °C for 1 h. The peritoneal cells were incubated on a plastic surface for 2 h and the non-adherent supernatant cells used in this assay. The parasites were prepared by standard methods[13]. 0.3-ml cultures were set up in flat-based microtitre trays[5,6] to provide a cell:parasite ratio of 2,500:1 for newborn larvae, 4,000:1 for infective larvae and 8,000:1 for adult worms. These cultures were incubated at 37 °C for 90 min before assessing the cell adherence to the surface of the parasites by microscopic observation. The end point of the assay was taken as the serum dilution in which 50% of the parasite's surface area was covered with cells. The adherent cells were identified by staining with Giemsa and with chromotrope R, the distribution being approximately 95% eosinophils and 5% mast cells. The points indicated represent the means of at least three experiments, each carried out in triplicate. The s.d. value at each point did not exceed 0.25 except with infective larvae on days 18 and 20, where the s.d.'s were 0.7 and 0.4, respectively. With newborn larvae the values were less than 0.25 except on days 18 and 20 where they were 0.4 and 0.6.

the serum of animals infected with the nematodes *Trichinella spiralis* and *Nippostrongylus brasiliensis* contain antibodies to nematode cuticle, each specific to the surface of a particular stage in the life cycle of the parasites and capable of mediating attack by inflammatory cells against the nematode surface.

The presence in the serum of rats of factors interacting with the surface of nematodes was assessed by studying the adherence to the worms *in vitro* of peritoneal cell populations obtained from normal August rats, as described previously[5,6]. The cell populations used in this study were obtained by incubating peritoneal cells on a plastic surface for 2 h at 37 °C and collecting the non-adherent cells which consisted of 60–80% eosinophils, 15–20% mast cells and 5–20% mononuclear cells. Initial studies showed that even in serum freshly obtained from uninfected rats, cells adhered to the surface of some but not all stages in the life cycle of the nematodes studied. In dilutions of fresh normal serum, cells adhered to infective larvae of *T. spiralis* and *N. brasiliensis* to a titre of 1/300 and to adult worms of both species to a dilution of 1/60, but did not adhere to the newborn larvae of *T. spiralis*, which is the stage shed by the viviparous mature females of this species. Adherence was markedly reduced after the normal serum had been inactivated by heating at 56 °C for 1 h or had been treated with zymosan (5 mg per ml serum; Zymosan A, Sigma)[7].

These results show that the surface of infective larvae and adult worms activate complement but that the cuticle of newborn *T. spiralis* larvae does not have this property. However, heat-stable factors which cause adherence of the eosinophil-rich cell population to all stages of these parasites occur in the serum of infected animals. Figure 1 shows the occurrence of these factors in the serum of rats infected orally with 4,000 *T. spiralis* infective larvae. The appearance and maximum titre of these factors were different for each stage of the parasite, suggesting the presence of antibodies specific for each life-cycle stage. Adsorption studies confirmed this. When serum taken from rats 14 d after infection was absorbed with either infective larvae or adult *T. spiralis*, the serum factor mediating cellular adherence to the homologous but not heterologous life-cycle stage was removed (Fig. 2). Similar adsorption and time-course studies have shown that the serum factor mediating cell adherence to *N. brasiliensis* infective larvae differs from that mediating adherence to adult *N. brasiliensis*[8]. Because of their intense specificity for each stage, we assume that these serum factors are antibodies.

Although antibodies to the surface of nematodes have frequently been described using such methods as immunofluorescence[9,10] or cell adherence[6,11,12], their significance in terms of the nature of the parasite surface and its role in the host–parasite reaction has not been assessed. Further studies using serum and cells from normal or infected rats showed that the adherence of cells in the presence of serum from infected animals may have a lethal effect on these parasites at least *in vitro*. *T. spiralis* newborn larvae were killed after incubation for 25–35 h in the presence of serum from either infected or uninfected donors (Table 1). Fresh normal serum did not affect the viability of the parasites even in the presence of cells. However, the lethal effect of cells and the serum factors from infected animals was greatly enhanced when freshly collected, rather than heat-inactivated, serum was used. Thus, complement seems to affect newborn larvae of *T. spiralis* only in the presence of antibody. Whether or not cells must adhere to produce damage has not yet been clearly determined; however, dead or dying larvae always had adherent cells, generally eosinophils. Preliminary experiments with purified cell suspensions implicate this cell type as an effector cell in causing damage to newborn larvae in this *in vitro* model. The poorer ability of the cells from infected animals to damage larvae may therefore be due to the lower proportion of eosinophil leukocytes in these cell suspensions. A percentage of infective larvae of *T. spiralis* and *N. brasiliensis* can also be damaged by incubation in fresh serum taken from infected animals together with peritoneal cells, but we have not yet fully defined the parameters of this reaction.

This study has revealed three features of the surface of nematode parasites. The surface of infective larvae and adults of *T. spiralis* and *N. brasiliensis* may activate complement, but the surface of newborn larvae does not have this property. During

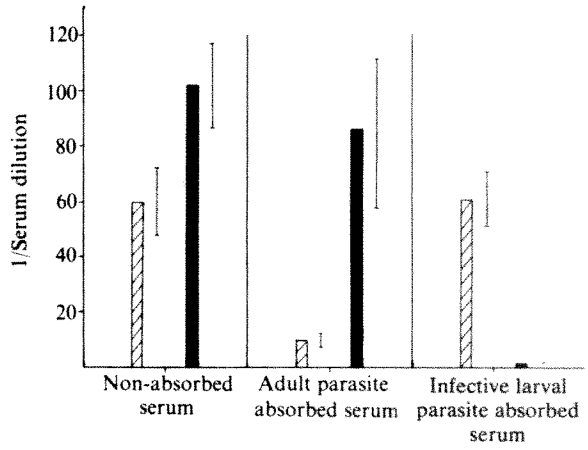

Fig. 2 Adsorption studies to show the specificity of the serum factors mediating cell adherence to *T. spiralis*. Serum obtained 14 d after infection with 4,000 *T. spiralis* infective larvae was assayed for its ability to mediate cell adherence before and after adsorption with adults (☐) or infective (■) larvae. An equal volume of freshly prepared parasites was added to a 1 in 3 dilution of the previously heat-inactivated serum and incubated at 37 °C for 90 min. The supernatant was then assayed as described in Fig. 1; s.d. values are expressed as bar lines.

infection, each stage in the life cycle of *T. spiralis* or *N. brasiliensis* stimulates antibodies which are specific for the surface of that stage only. Finally, in the presence of these antibodies, the parasites are vulnerable to attack and may be killed by peritoneal cell populations enriched in eosinophils, especially in freshly collected serum. Variations in the interaction between the host and different parasitic stages of an infecting nematode are likely to be due in part to differences in the interaction of these stages with complement, antibody and inflammatory cells.

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Interferon inhibits the release of plasminogen activator from SV3T3 cells

INTERFERON, as well as inducing an antiviral state, can modify certain other cell characteristics. For example, it has been shown to inhibit cell proliferation[1–4] and release of C-type virus[5,6], and to enhance the expression of H-2 antigens[7,8] and the amount of glycoprotein on the cell surface[9]. These effects might be explained by decreased release of cell-surface components into the medium. Similarly, the expression of a glycoprotein on the cell surface has been related to the growth state of cells[10], which can be explained in part by differential release[11,12]. We have followed up this suggestion by examining the effect of interferon on the release of plasminogen activator (PA) by a line of simian virus 40 transformed Swiss 3T3 (SV3T3) cells. Our results, reported here, indicate that interferon inhibits the release of PA by SV3T3 cells.

First, to establish the sensitivity of SV3T3 cells to the anticellular activity of interferon, we grew cells in the presence of 50, 500, or 5,000 units ml^{-1} of a crude preparation of interferon[13]. Cell numbers (day 3) were reduced by 15, 40, and 50%, respectively, by these doses. These results were confirmed using the semi-purified mouse L cell interferon (3×10^7 NIH units per mg protein, obtained from Dr K. Paucker[14]) which was used for the rest of the study. Similar observations have been reported for several other cell types which respond to the anticellular properties of interferon[1–4].

The release of PA by SV3T3 cells is independent of cell density[15]. To determine whether interferon alters this pattern, we grew SV3T3 cells in the presence of interferon for 24 h and measured the PA released into a fresh serum-free harvest fluid (HF) during the next 10–12 h. After collection of the HF, the cells were lysed with Triton X-100, and lysate PA content also determined. Treatment with 50, 100, or 500 NIH reference units interferon ml^{-1} resulted in 37, 54, and 58% reductions, respectively, in the amount of PA released into the HF (significant by Students' t test, $P < 0.005$) (Table 1*a*). In contrast, cell lysate PA activity was not significantly reduced. In two other experiments, up to 1,000 units interferon ml^{-1} did not inhibit PA release any further. Interferon doses higher than 200 units ml^{-1} have resulted in significant decreases in cell lysate PA in some experiments. Direct addition of interferon (up to 500 units ml^{-1}) to the PA assay, or preincubation of control PA samples with interferon for 1 h at 37 °C, did not decrease the assayable PA.

In spite of the relative purity of the interferon used, the participation of residual impurities was considered. Pretreat-

Table 1 The effects of interferon and cycloheximide on PA activity and protein synthesis

	% of control units PA†		^{3}H-amino acid incorporation‡ (c.p.m. mg protein^{-1})
	HF	Lysate	
a. Interferon* (Units ml^{-1})			
0	100±4	100±2	5,994±177
50	63±5	97±3	6,308±171
100	46±4	88±4	6,395±130
500	42±8	106±6	5,646±166
b. Cycloheximide§ (μg ml^{-1})			
0	100±4	100±2	3,286±317
0.1	64±5	96±3	1,934± 35
0.2	31±1	64±4	1,044± 23
0.5	19±1	40±1	650± 26

*Interferon was assayed in 3T3 cells by a neutral red dye uptake method patterned after that of Finter[26] using vesicular stomatitis virus as the challenge virus. Units are expressed as reference units based on NIH reference standard G-002-904-51.

†Cells were plated at 5×10^4 cells per 35-mm plastic petri dish (Falcon) in Dulbecco's modification of Eagle's minimal essential medium (Gibco) supplemented with fetal calf serum to a final concentration of 5%. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air for 18 h before addition of interferon with medium change. Cell counts were performed in duplicate on cells resuspended by trypsinisation. Cells were counted using a haemocytometer. For preparation of PA samples, cells were grown to a density of 2×10^5–3×10^5 per dish, switched to serum-free medium ± interferon, and the HF collected after an additional 10–12 h of incubation at 37 °C Triton X-100 cell lysates were prepared, and HF and lysate samples assayed for PA activity using ^{125}I-fibrin as the substrate as previously described[27], with slight modifications. The assay mixture contained. 0.1% Triton X-100 in 0.1 M Tris-HCl, pH 8.1, 5–10 μg of purified human plasminogen[28], and HF or lysate sample in 1 0 ml final volume One unit of PA is the amount of enzyme generating enough plasmin to catalyse the hydrolysis of 10% of the total fibrin substrate in 4 h at 37 °C. Plasminogen blanks were subtracted before calculation of the number of units, and data were normalised for cell number or for total protein in Triton X-100 lysates, as determined by the procedure of Lowry *et al.*[29]. Each value is the mean of data from three separate experiments. Each experiment was carried out in duplicate, and each resulting sample assayed two or three times. Data are expressed as % of control values to facilitate comparisons of data derived from separate experiments. Data are shown ± s.e.m.

‡^{3}H-amino acid incorporation was measured at the end of the HF collection using dishes treated in parallel with those used to measure PA. For labelling, cells were washed twice with 37 °C Hank's balanced salt solution (HBSS) containing appropriate doses of interferon or cycloheximide. Cells were labelled for 1 h with 2.5 μCi ^{3}H-L-amino acid mixture (New England Nuclear) in 1 0 ml of HBSS containing interferon or cycloheximide After 1 h the medium was removed, and the cells washed three times with warm HBSS, extracted three times with cold 5% trichloroacetic acid (TCA), and solubilised in 0.6 ml of 0.5 M NaOH. Fetal calf serum (20 μl) was added to duplicate 0.2 ml portions of the solution to serve as unlabelled carrier protein, and the samples neutralised with 4 M HCl. Neutralised samples were precipitated with 2.0 ml of cold TCA and stored at 4 °C overnight. TCA precipitates were collected on Whatman GF/C filters and the filters washed exhaustively with cold 5% TCA, dried, and counted in 10 ml of Omnifluor (NEN) toluene. Data were normalised for protein content of the NaOH solutions as described above and represent mean values ± s.e.m. of duplicate determinations from each of two cultures.

§Cycloheximide (Sigma) was added at the beginning of HF preparation for PA studies.

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ment of interferon (100 units ml^{-1}, final concentration) for 1 h at 37 °C with a 1:200 dilution of rabbit anti-mouse interferon serum (titre 4,000, Reference Reagents Branch, NIH) inactivated its antiviral activity, as well as its inhibition of PA release (inhibited by only 13%). In parallel experiments, interferon treated with preimmune serum, or with diluent, retained activity, inhibiting PA release by 46 and 48% respectively. Column void material from an anti-interferon globulin affinity column used for interferon purification[14] did not inhibit PA release even when used at a pre-column equivalent of 10,000 units of interferon ml^{-1}. Heating interferon at 60 °C for 1 h destroyed its effect on PA release, but dialysis at 4 °C for 24 h did not. These properties of mouse interferon are consistent with those reported by Gresser *et al.*[1], and indicate that the observed inhibition of PA release is due to interferon itself.

Interferon does not cause accumulation of PA in SV3T3 cells (Table 1*a*), and at high doses may decrease cellular PA (data not shown). Further, high doses of interferon inhibit protein synthesis in mouse L cells[16]. Thus, the possibility that the effect of interferon on PA release might be due to a generalised inhibition of protein synthesis in SV3T3 cells was considered. Doses of interferon up to 500 units ml^{-1} had no measurable effect on incorporation of ^{3}H-amino acids in SV3T3 cells (Table 1*a*). The total amount of radioactivity (c.p.m.) incorporated was within the linear range for the experimental conditions used. Under similar conditions, cycloheximide (0.1–0.5 μg ml^{-1}) caused a marked, dose-dependent inhibition of ^{3}H-amino acid incorporation (Table 1*b*). Both HF and cell lysate PA activities were also inhibited by cycloheximide. Thus, although inhibition of protein synthesis with cycloheximide causes decreased PA activity in SV3T3 cells, the interferon-mediated inhibition of PA release cannot be explained in terms of a general inhibition of protein synthesis.

Our data suggest that interferon inhibits some aspect of the process of release of PA. Although the mechanism of PA release is not clear, a secretion mechanism has been suggested[17,18]. The firm membrane association of PA (refs 19–21), and its enrichment in plasma membrane fractions of chicken and hamster cells[21], suggest to us that PA may be released through the process of membrane shedding, such as that described for histocompatibility antigens[22,23] and certain membrane-associated glycosyl transferases[24,25]. Thus, inhibition of PA release could be a direct effect of interferon on shedding. Alternatively, since PA release is dependent on cell density in 3T3 cells[15], the interferon effect on SV3T3 cells may be indirect, because of inhibition of cell proliferation. Neither do we yet know whether the interferon effect extends to the release of other cell components. Experiments are in progress to examine these questions.

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Different rules govern help for cytotoxic T cells and B cells

RECENT experiments with radiation chimaeras[1,2] indicate that T cells differentiating in an H-2D compatible, but H-2I different, thymic environment are completely unresponsive to vaccinia virus. The explanation favoured[2] for this surprising finding is that participation of helper T cells[3] (TH) is an absolute requirement for the generation of virus-immune cytotoxic T cells (Tc). No response is seen because the TH populations are 'locked in' to recognising the spectrum of H-2I antigens presented on radiation-resistant thymic cells[2]. They cannot, therefore, interact with the Tc precursors which are genetically different in the I region. The result is a total failure of responsiveness. We report here that we have tested this concept further by stimulating acutely tolerised[4,5] T-cell populations in H-2I different, but H-2D compatible virus-infected recipients. Our results indicate that there is apparently no need for participation of TH populations restricted to the H-2I region specificities expressed in the recipient environment. This differs from the situation for TH-B-cell collaboration[4,5], and suggests that help for the Tc response operates directly between T-cell subsets.

An elegant analysis of genetic requirements for T-cell–B-cell collaboration has been made by Sprent[4,5], who used negative selection protocols to remove alloreactive lymphocytes and then tested for TH to sheep red blood cells in irradiated recipients compatible for different regions of the H-2 gene complex. The

Table 1 H-2 haplotypes of mice and target cells

	K	A	B	J	E	C	S	D
B10.Br, C3H, CBA/J, L cell	k	k	k	k	k	k	k	k
BALB/c, B10.D2, P815	d	d	d	d	d	d	d	d
C57BL/6, BALB/B, MC57G	b	b	b	b	b	b	b	b
B10.A	k	k	k	k	k	d	d	d
B10.A(2R), 2RSV	k	k	k	k	k	d	d	d
B10.A(3R)	b	b	b	b	k	d	d	d
B10.A(5R), 5RSV	b	b	b	k	k	d	d	d
BALB/G, B10.HTGSV	d	d	d	d	d	d	d	b

SV, Target cells transformed with SV40 virus.

conclusion reached from these studies, and from experiments with chimaeras, is that the macrophages in the stimulator environment and the B cells to be helped must share I-region determinants. Furthermore, as for the Tc response to vaccinia virus[2], differentiating TH precursors only operate in the context of H–2I antigens encountered previously in radiation-resistant thymus[5].

Our intention was to determine whether the same spectrum of interactions applies to the generation of virus-immune cytotoxic T cells. We know that a detectable Tc response only occurs following exposure to virally modified macrophages expressing the H–2K or H–2D phenotype encountered previously in thymus. Does the postulated interaction of TH and Tc[2] cells also require involvement of an infected macrophage presenting, as for T–B collaboration[4,5], the same I-region determinants expressed on radiation-resistant thymus epithelium?

Similar negative selection procedures[4,5] and H–2 haplotype combinations were thus used to analyse the postulated requirement for TH in the Tc response to vaccinia virus. B10.D2 spleen and lymph node populations were first filtered through an irradiated (BALB/c×C57)F$_1$ environment, and thoracic duct lymphocytes were then stimulated with vaccinia virus[6] in B10.A(5R) recipients, which differ from H–2K to I–E. Contrary to expectations, a strong virus-specific Tc response was seen at H–2D^d (Table 2). This cannot be attributed to operation of radiation-resistant TH in the recipients, as the chimaera cells were exposed to virus in the same way but were unresponsive[2]. Stimulation in the absence of other than H–2D end (I–C to –D) homology with the recipient was confirmed in a further experiment (Table 3), where (C3H×BALB/c)F$_1$ T cells were used to minimise the possible involvement of some form of allogeneic effect[7] operating at I–J or I–E (Tables 1 and 2). Furthermore, the addition of lymphocytes capable of mediating allogeneic effects during the sensitisation process (unpublished data) has not, to date, caused any significant enhancement of cytotoxicity in situations where there is a low Tc response[8] to vaccinia virus.

A comparable lack of requirement for I-region homology between T-cell and irradiated recipient was also found for another H–2 haplotype combination (Table 4). However, in one case (Expt 2, Table 4), there was evidence of either residual alloreactivity or generation of a virus-immune cytotoxic T-cell response operating across the H–2 barrier. The second of these possibilities has now been confirmed[9].

Apparently, virus-specific cytotoxic T cells do not need to share I-region genes with the recipient in which they are stimulated, although the presence of the relevant H–2K- or H–2D-encoded antigens is essential (Tables 2 and 4). Thus, if TH cells are involved in the Tc response to vaccinia virus, the mechanism seems to be somewhat different from that defined for TH–B-cell–macrophage interactions, where I-region restriction seems to be absolute when analysed by similar experimental protocols[4,5]. Perhaps helper T cells involved in the virus-immune cytotoxic response are cross-reactive for infected macrophages expressing I-A^d and I-A^b (Tables 2 and 3), or I-A^d and I-A^k (Table 4). The latter is, however, the same combination for which a total failure of the Tc response to vaccinia virus was found in the chimaera experiments[2]. An alternative interpretation is that the postulated interaction between TH and Tc is quite independent of the recipient macrophages and operates essentially between T-cell subsets. Recognition may, for example, be specific for T-cell receptor idiotype[10]. We must also consider the possibility that the influence of I-region genes on the virus-immune Tc response is mediated other than through T-cell help at the time of antigen stimulation, perhaps reflecting

Table 2 Negatively selected T cells of the I-A^d genotype generate a strong cytotoxic response when stimulated in an H–2D^d-compatible recipient expressing I-A^b

Population stimulated	950R recipient	% specific ^{51}Cr release (25·1) 5RSV Vacc.	5RSV N	P815 Vacc.	P815 N	MC57G Vacc.	MC57G N	L cell Vacc.
B10.D2 in C57	B10.A(5R)	69	8	28	–	0	0	16
(BALB/c×C57)F$_1$	B10.A(5R)	85	8	33	4	28	0	19
Unirradiated controls	(BALB/c×C57)F$_1$	57	11	30	4	23	13	15
	CBA/J	26	23	0	0	0	14	52

B10.D2 spleen and lymph node cells were inoculated intravenously (2 0–4.0×10^8 cells per mouse) into 10 irradiated (950R, 24 h) (BALB/c×C57BL/6)F$_1$ recipients, and negatively selected T-cells were drained from thoracic duct over the following 18–42 h (ref. 4). Approximately 4.0×10^7 lymphocytes were recovered from the 10 filter mice. These cells were then transferred to two irradiated receipients. Controls were unirradiated mice, or 950R recipients given 6.0×10^7 spleen and lymph node cells. All mice were injected intravenously with 5.0×10^6 plaque-forming units of vaccinia virus at 3 h after cell transfer, and spleen cells were tested for cytotoxicity 6 d later in a standard ^{51}Cr release assay (ref. 11) using vaccinia-infected (vacc.) or normal (N) target cells. All values given are for specific ^{51}Cr release, relative to incubation in medium alone. A strong cytotoxic T-cell response is seen at H–2D^d (5RSV) but not at H–2K^b (MC57G)

Table 3 Stimulation at H–2D^d in the absence of possible allogeneic effects

Population stimulated	950R recipient	% specific ^{51}Cr release (50:1) 5RSV Vacc.	5RSV N	MC57G Vacc.	MC57G N	P815 Vacc.	P815 N	L cell Vacc.	L cell N
(C3H×BALB/c)F$_1$ in (CBA×C57BL/6)F$_1$	B10.A(5R)	61	12	10	6	23	1	–	–
	B10 A(3R)	59	0	11	0	21	0	2	–
Unirradiated controls	B10.A(5R)	71	10	57	0	26	1	16	116
	C57BL/6	67	11	64	2	9	6	25	23
	B10 A	49	11	5	3	19	1	81	11
	B10 D2	53	10	9	5	38	1	11	16
	B10.Br	11	7	5	0	7	4	77	8
	BALB/c	36	6	5	0	28	3	15	10
	(C3H×BALB/c)F$_1$	38	12	17	9	19	1	55	12
	(BALB/c×C57BL/6)F$_1$	56	6	31	0	28	1	13	13

(C3H×BALB/c)F$_1$ spleen and lymph node cells were filtered through 20 irradiated (CBA/J×C57BL/6)F$_1$ recipients and then stimulated with vaccinia virus in 950R recipients, as described in Table 2

Table 4 Stimulation of negatively selected T cells in recipients which differ from K to I–J but share D-end antigenic specificities

			% specific ^{51}Cr release (25 1)			
			5RSV		L cell	
Expt	Population stimulated	950R recipients	Vacc	N	Vacc.	N
	BALB/c in CBA	A/J	32	–	1	4
1	(BALB/c×CBA)F_1	BALB/c	68	11	7	0
	unirradiated CBA/J		6	8	75	0
	BALB/c in CBA	B10.A	40	7	21	–
2	(BALB/c×CBA)F_1	(BALB/c×CBA)F_1	61	4/	73	11
	(BALB/c×CBA)F_1	BALB/c	28	7	4	3
	(BALB/c×CBA)F_1	CBA	2	0	68	7

physiological mechanisms functioning during the process of lymphocyte differentiation in thymus[1,11]. Is help involved in the generation of the Tc repertoire?

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Suppression of human T-cell colony formation during pregnancy

THE human fetus, as a natural allograft, escapes destruction by the maternal immune system. As a possible mechanism, Alexander[1] proposed a rapid shedding of transplantation antigens by embryonic cells, resulting in a blindfolding of the otherwise fully functional maternal immune system. Other investigators have reported a suppressive activity of lymphoid cells from human cord blood on the proliferation of maternal lymphocytes[2,3]. Suppressor T cells are induced by α-fetoprotein in mice[4], and the elevated levels of α-fetoprotein during pregnancy have been shown to exert an immunosuppressive influence in mice[5,6] and in man[7]. Olding[8] demonstrated a soluble factor from mitogen stimulated lymphoid cells of human newborns suppressing the proliferation of maternal lymphocytes. These observations point out that the embryo might protect itself by release of soluble factors, acting either themselves as lymphocyte suppressive agents, and/or by the induction of suppressor cells. However, no direct evidence has so far been presented to show the presence and action of T-cell suppressor cells in the immune system of pregnant women. Such an experiment would have to use components of the immune system of pregnant women solely, and therein demonstrate an immune suppression as compared to non-pregnant individuals. We present here results indicating that the ability of T cells from pregnant women to proliferate in soft agar cultures is strongly inhibited, regardless of the source of the serum being used in the assay, thus pointing rather to a cell-mediated suppressor mechanism.

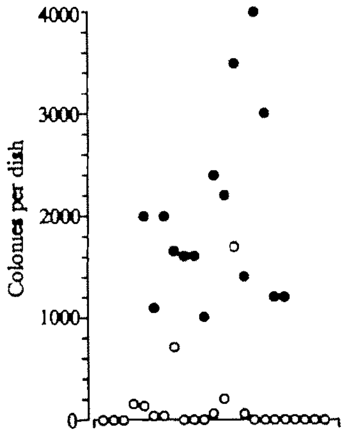

Fig. 1 3×10^6 lymphocytes were incubated overnight with PHA (4 μg ml^{-1}) in 5 ml RPMI 1640 containing 15% autologous serum. After 15–18 h, 2×10^5 cells were seeded into a RPMI-soft agar medium, containing the same serum, and incubated for 5 d at 37 °C and 7% CO_2. The number of T-cell colonies from non-pregnant donors (●) ranged from 1,000 to 4,000. The pregnant women (○) had a strongly impaired colony formation

Twenty healthy pregnant women aged 20–34 yr and between the 10th and 41st weeks of gestation, gave their informed consent to *in vitro* analysis of cellular immune functions. Venous blood was collected in syringes containing RPMI 1640 and heparin, and the lymphoid cells were isolated by the Ficoll–Hypaque technique[9].

Phytohaemagglutinin A(PHA) and concanavalin A(ConA) stimulation *in vitro* was performed according to Cunningham-Rundles[10]. T cell-quantitation[11] was performed in each case, as well as the T-cell colony test (TCC-test[12,13]). For the TCC-test, purified PHA (Wellcome, catalogue no. HA 17) instead of PHA-M was used. Thus we could obtain 1,000–4,000 colonies per dish, seeding 2×10^5 lymphocytes into the dishes, from healthy non-pregnant donors. Of the cells obtained from the colonies, 80–90% formed E-rosettes, characteristic for T cells.

Table 1 shows the T-cell proportions and the lectin stimulation test in the pregnant women compared with a normal population. No significant change in the T-cell proportions and no marked inhibition of lectin stimulation was seen in the pregnant women. Figure 1 shows the TCC-test in the pregnant women, compared with the activity in 15 healthy non-pregnant donors. In 61% of the cases the pregnant women were completely unable to form colonies. In 30% of the cases, the colony forming activity was reduced to less than 5% of the activity in the control donors, the normal mean value being 2,000, (standard derivation, s_x, 900) per dish. If the cells from the pregnant women were incubated in a pooled human serum in which the control cells gave normal colony counts, no improvement of the colony forming activity could be observed (Fig. 2*a*).

Lymphocytes of healthy donors cultivated as an ABO compatible system in the presence of serum from the pregnant women (Fig. 2*b*) gave normal colony counts. Hence we assume that the factor depressing the clonal proliferation of T-cells in

Table 1 T-cell proportions and lectin stimulation test in pregnant and non-pregnant women

	Pregnancy		Control	
	$\bar{x}$	s_x	$\bar{x}$	s_x
T cells (%)	68.5	10.3	72.5	8.3
PHA stimulation (c.p.m.)	32,626	16,060	21,590	6,171
ConA stimulation (c.p.m.)	11,122	5,075	18,620	11,206

5×10^4 lymphocytes were incubated in 150 μl RPMI 1640 with 15% pooled human serum and 60 μg ml^{-1} ConA or 20 μg ml^{-1} PHA-P. After 96 h at 37 °C, 0.2 μCi ^{3}H-TdR (6.4 Ci mmol^{-1}) was added. After 24 h the cultures were collected using a multiple sample harvester, and counted in a scintillation counter.

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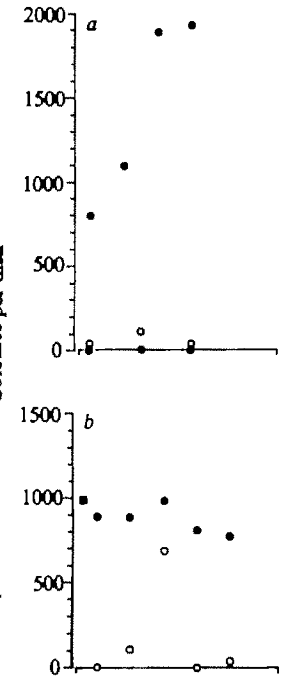

Fig. 2 *a*, T-cell colonies from non-pregnant donors in pooled serum (●), from pregnant women, incubated in the same serum pool (◐) and in autologous serum (○). Substitution of the serum from the pregnant women by normal serum caused no improvement of the colony formation. *b*, T-cell colony count from one non-pregnant cell donor after incubation in autologous serum (■), and in serum of ABO-compatible pregnant women (●). The T-cell colony formation from the same pregnant women in their own serum served as a control (○) No significant depression of normal T-cell colony formation was seen in serum from pregnant women.

this system is cellular, rather than humoral. The presence of a factor in the blood of pregnant women able to suppress the proliferation of T lymphocytes may, considering the report of Olding[8], reflect the ability of the fetus to defend itself against the maternal immune system.

The failure of lectin stimulation analysis to show any significant change in the pregnant women indicates that the TCC-test has a much higher sensitivity in detecting factors inhibiting T-cell proliferation.

The kinetics of the suppressor phenomenon described here, are now being investigated. Preliminary results have shown that the immune depression described above is seen as early as 6–10 weeks after onset of pregnancy, and is abolished between the 35th week of gestation and the sixth week after delivery.

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Catecholamines of supposed inhibitory hypophysiotrophic function suppress action potentials in prolactin cells

THE anterior lobe of the pituitary gland has a uniquely important function in the endocrine system, for its six different endocrine cell types constitute the major endocrine link between the brain and the rest of the body. The brain is known to control this remarkable composite gland through specialised neurones lodged in the hypothalamus that elaborate and release hypophysiotrophic factors; these are carried by the hypophysial portal blood vessels to act on their respective target cells through stimulation or inhibition of secretory activity[1,2]. However, the way in which these factors act at the cellular level is uncertain, although discharge of the various hormones of the anterior pituitary lobe is known to show calcium dependence and can be induced by depolarising concentrations of potassium[3–5]; this pattern of endocrine behaviour was first recognised in adrenal chromaffin cells[6] and is consistent with the calcium hypothesis of stimulus–secretion coupling[7], which, in chromaffin and some other cells, is associated with electrical excitability[8–13]. It is therefore of much interest that normal gland cells of the anterior pituitary lobe[14,15], like their neoplastic counterparts[16–18], have been found to generate action potentials with a calcium component indicative of influx of calcium ions; furthermore, the frequency of action potentials can be increased in some of these cells by thyrotropin releasing factor (TRF)[14]. This has prompted the suggestion that it may be through the initiation or modulation of action potentials that the brain, through the hypophysiotrophic factors, regulates secretion in the anterior pituitary[14]. However, the evidence which supports this scheme is scanty. The experiments with TRF were carried out on disaggregated anterior pituitary lobes of rats which contain six different endocrine cells, and the method did not allow identification of the cells stimulated by TRF; thus, it could only be supposed that they were the appropriate target cells[14]. Clearly, it is essential that evidence be obtained on the electrical behaviour of normal anterior lobe cells of defined type, and the effects thereon of specific and relevant hypophysiotrophic factors. Here we report that normal prolactin cells generate spontaneous action potentials which are suppressed by hypothalmic catecholamines known to inhibit prolactin secretion.

In mammals, the intermingling of the various endocrine cells in the anterior lobe of the pituitary impedes the correlation of electrical behaviour with cell type. However, in teleost fishes, the various anterior pituitary cells are segregated by functional type into distinct regions. In particular, the prolactin cells, which are most appropriate for our purpose, are grouped in a distinct lobe (rostral pars distalis) which is easy to identify and excise[19–21]. Moreover, teleostean prolactin cells are a suitable model as they behave like their mammalian counterparts in important respects: their secretory activity *in vivo* is subject to inhibitory hypothalamic control apparently exerted by hypophysiotrophic catecholamines; and *in vitro*, when this control is absent, secretion increases greatly but can be restrained by catecholamines[19–22]. It follows that if action potentials are intimately involved in regulating secretion, then prolactin cells should show spontaneous action potential activity *in vitro* and this should be inhibited by catecholamines. Our experiments confirm these expectations and demonstrate that the action

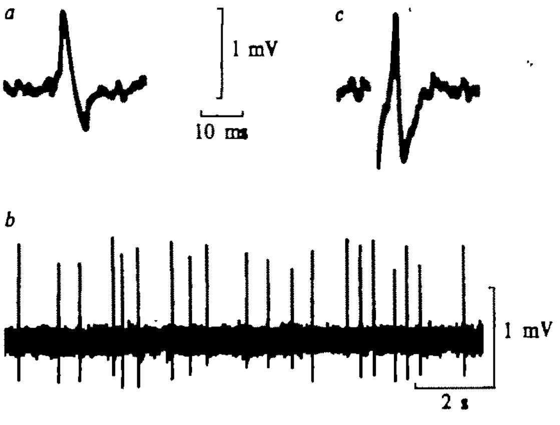

Fig. 1 Action potentials in prolactin cells. *a* and *b*, Spontaneous action potentials recorded extracellularly (with a microsuction electrode[14]) at fast and slow sweep speeds from the same cell, to demonstrate the shape of individual action potentials (*a*) and pattern of spontaneous activity (*b*). *c*, Evoked action potential from a second cell elicited by an outward (depolarising) current pulse of 3.5 nA passed through the extracellular recording electrode. The frequency of spontaneous action potentials in this cell was low (0.08 Hz). The recording solution contained (in mM) NaCl, 183; KCl, 5; $CaCl_2$, 2; glucose, 5.5; HEPES, 5 (*p*H 7.1) with 1 mg ml^{-1} bovine serum albumin (BSA). Experiments were done at 17–20 °C. Top calibrations apply to both *a* and *c*. The cells used for electrophysiological study were prolactin cells in primary culture obtained as follows. Pituitary glands were removed immediately after decapitating live, mature (about 30 cm) *Alosa pseudoharengus* (10–20 specimens per experiment). The anterior one-half to two-thirds of rostral pars distalis was excised from each, pooled and mechanically disaggregated by shearing with siliconised Pasteur pipettes with flame-polished tips of inner diameter ≤1 mm. Disaggregation medium was 3 ml Ham's F-10 (Gibco) supplemented with 15% horse serum, 2.5% fetal calf serum, 35 mM NaCl, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin, and saturated with 5% CO_2 – 95% O_2 at room temperature. Dispersed cells were centrifuged at 100*g* for 8 min and the pellet resuspended in 2 ml recording solution (see above). To remove debris, suspended cells were layered on 5 ml recording solution containing 4% BSA and spun down at 100*g* for 8 min. Sedimented cells were resuspended in supplemented Ham's F-10 and placed in Falcon dishes (35 mm) coated with poly-L-lysine (Sigma type V). Dishes, each containing cells from two pituitaries in 2 ml medium, were maintained at room temperature and generally used for experiment within 10 d.

potentials involve a calcium component which probably provides a stimulus to secretion.

The fish used was the alewife (*Alosa pseudoharengus*); the pituitary gland of this species has a large and clearly defined rostral pars distalis shown to consist essentially of a mass of large prolactin cells containing prominent secretory granules, the only other endocrine cells present being small ACTH cells arranged in a thin layer along the posterior border[33]. We avoided these ACTH cells by using only the anterior one-half to two-thirds of the rostral pars distalis, which we disaggregated and maintained in culture (see legend to Fig. 1). The dispersed cells were observed under phase contrast at high magnification (×800) and records were obtained from those whose large size and granular content indicated them to be prolactin cells.

Most of the cells monitored showed spontaneous action potential activity (Fig. 1*a*). Of >100 spontaneously active cells, almost all fired at random intervals (Fig. 1*b*), but a few fired in a bursting pattern. Action potentials could also be induced, or their frequency increased, by outward (depolarising) current pulses passed through the electrode (Fig. 1*c*). When driven by current pulses, cells sometimes fired at frequencies in excess of 25 Hz. The average rate of spontaneous discharge, however, was 1 Hz (0.8 ± 0.1 Hz, mean ± s.e.m., $n = 23$, records selected to avoid influences of drugs).

Tetrodotoxin (TTX) reversibly depressed the amplitude of the action potentials (Fig. 2*a*, *b*), indicating that they result mainly from an increase in membrane conductance to sodium ions[34]. However, a calcium component was also present, as blockade of potential-dependent potassium conductance by the addition of tetraethylammonium (TEA) to the TTX-containing solution resulted in large regenerative potentials that were reversibly suppressed by replacing calcium in the bathing solution with manganese (Fig. 2*c*, *d*). The action potentials thus involve inward currents of both sodium and calcium ions (see refs 35, 36).

The catecholamines dopamine and noradrenaline, which are the principal factors in hypothalamic extracts capable of inhibiting secretion from isolated prolactin cells[37,39], had inhibitory effects on spontaneously firing prolactin cells and either slowed or arrested the discharge of action potentials. Arrest was the most common response when these substances were used in a concentration of 10^{-6} M. It occurred within a few seconds and often persisted for many seconds after the catecholamine had been withdrawn (Fig. 3*a*, *b*). We have not attempted to determine minimal effective concentrations but have noted that concentrations as low as 10^{-8} M can slow impulse discharge (Fig. 3*c*). When discharge persisted, albeit at reduced frequency, during exposure to dopamine or noradrenaline, the amplitude of the action potentials was unaltered. The catecholamines therefore seem to inhibit the mechanism which initiates the action potentials (the 'generator potential') and not the potential-dependent conductances that underlie these spikes.

Our experiments thus provide new information on the control of anterior pituitary secretion: (1) They indicate that normal prolactin cells, in conditions in which they secrete spontaneously, have spontaneous action potentials; (2) they demonstrate that these action potentials, although mediated mainly by sodium, involve inward calcium movement which, from evidence on many secretory systems[7,40] including prolactin and other anterior pituitary cells[3–5], should provide a stimulus to secretion; (3) they show that putative inhibitory hypophysiotrophic factors reduce this electrical activity. Together, these findings strengthen the hypothesis that hypophysiotrophic factors control secretion in the anterior pituitary gland by

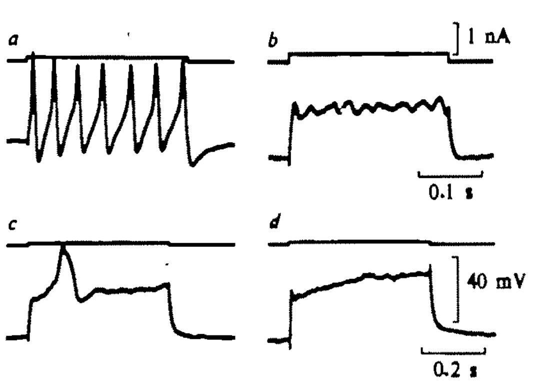

Fig. 2 Ionic basis of action potentials in prolactin cells. Intracellular recordings from two different cells showing the effect of TTX (*a*, *b*) and of replacement of Ca^{2+} by Mn^{2+} (*c*, *d*) *a*, Repetitive action potentials evoked by a single outward (depolarising) current pulse passed through the recording electrode. *b*, Response of the same cell during exposure to TTX (2×10^{-6} M) applied by a delivery pipette[14]. The action potentials are virtually abolished *c*, Response of a second cell to a depolarising pulse while bathed in a solution containing TTX (5×10^{-6} M) with the further addition of TEA (10 mM). A prominent regenerative potential is evident. *d*, Response of the same cell when perfused (by drug delivery pipette) with the same medium except that Ca^{2+} was replaced by Mn^{2+}. The regenerative response is abolished. Top trace in all records indicates current passed and zero level of membrane potential. Calibrations for current and potential apply to all four sets of records. Intracellular recording was done by conventional techniques using glass microelectrodes filled with 4M potassium acetate, *p*H 7.1, with resistance 60–100 MΩ. A bridge circuit was used for passing current. To facilitate intracellular recording the Ca^{2+} concentrations were increased to 10 mM throughout except in *d*, where it was replaced by 10 mM Mn^{2+}.

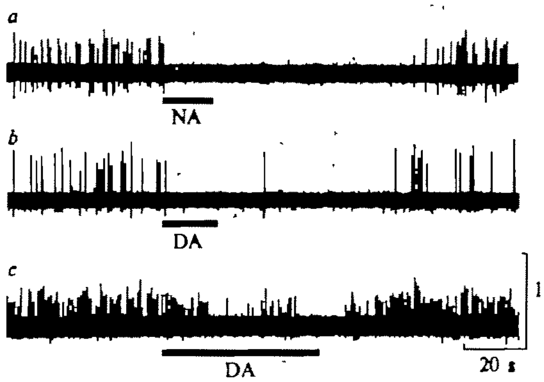

Fig. 3 Inhibition of spontaneous action potentials in prolactin cells by catecholamines. Catecholamines (in recording solution) were applied to the cells by drug delivery pipette for periods indicated by bars under the records. *a*, Arrest of spontaneous discharge by noradrenaline (10^{-6} M NA). *b*, Similar effect, in a second cell, produced by dopamine (10^{-6} M DA) *c*, Slowing of spontaneous discharge, in a third cell, by a lower concentration of dopamine (10^{-8} M). Similar responses were obtained in 32 of 34 cells tested.

affecting action potential activity in their target cells. Finally, in common with others[19–21], we consider that the teleost pituitary, because of the segregation of cell types, offers unique advantages for the study of hypophysial function.

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Neurone-specific enolase is a molecular marker for peripheral and central neuroendocrine cells

NEURONE-SPECIFIC ENOLASE (NSE) is the most acidic brain isoenzyme of the glycolytic enzyme enolase (EC4.2.1.11) and has been shown to be homologous to the 14-3-2 protein isolated from bovine brain by Moore[1–3]. Whereas NSE is exclusively localised in neurones in mammalian nervous tissue[4,5], another brain enolase isoenzyme termed non-neuronal enolase (NNE) is localised in glial elements[5]. As NSE and NNE are structurally, functionally and immunologically distinct isoenzymes that represent separate gene products[6,7], they are useful markers for cell classes in the nervous system. Although NNE is probably identical to liver enolase, NSE has to date been considered to be localised in neurones. We now report that NSE is also present in peripheral and central neuroendocrine cells, also termed amine precursor uptake and decarboxylation (APUD) cells[8–10]. Immunocytochemistry using the unlabelled antibody enzyme method of Sternberger[11] demonstrates that APUD cells in both laboratory rat and rhesus monkey (*Macacca mulatta*) stain positively with NSE antiserum. Using a sensitive radioimmunoassay (RIA) for NSE, it is possible to confirm their localisation in adrenal gland and to support the finding in other APUD-cell-containing tissues in rat, monkey and man. The results in human tissues suggest that the immunocytochemical localisation in rat and monkey will be valid for man and prove useful in the study of human diseases involving the APUD cell class.

Antisera to purified neurone-specific enolase isolated from rat (NSE-R) and human (NSE-H) brain were prepared in New Zealand white rabbits as previously described[12]. The sera were not reactive with NNE. Antiserum to NSE-H reacts identically with monkey and human brain extracts, indicating that this serum cross-reacts quantitatively with monkey NSE (NSE-M). Animals were anaesthetised and perfused through the aorta first with normal saline and then with 4% paraformaldehyde–1% glutaraldehyde–0.2% picric acid in 0.1 M sodium acetate buffer, *p*H 6.0, with 1.5% sucrose. Tissues were fixed for 2–6 h and washed overnight. Monkey tissue was cut into 10–25 μm thick sections on a vibratome or cryostat; rat tissue was imbedded in polyester wax[13] and cut at a thickness of 4–8 μm. The rest of the staining proceeded according to the unlabelled antibody enzyme method[11]. Preimmune serum and NSE-absorbed serum served as controls.

Stained sections revealed that NSE antiserum reacts specifically and uniformly with the APUD series of neuroendocrine cells[8–10] in each gland or tissue (Table 1). Both rat and rhesus monkey gave identical results. In pancreas, the cells of the islets of Langerhans exhibit positive staining, in contrast to the surrounding exocrine acinar and epithelial cells, which do not stain (Fig. 1*a*). All islet cells stain positively, although there is some variation in intensity among cells. NSE staining in the adrenal gland (Fig. 1*b*) clearly delineates the positive chromaffin cells of the medulla from the non-staining cortex. NSE-positive cells in the thyroid (Fig. 1*c*) are compatible in position and number with parafollicular calcitonin (CT) cells, whereas the thyroid follicular cells are NSE negative. Likewise, pinealocytes in the pineal and cells of the pituitary, especially those of the pars intermedia, all stained for NSE. Interstitial and epithelial cells remained unstained. In the hypothalamus, neuroendocrine cells contain NSE as do all neurones in the central and peripheral nervous system.

To test the hypothesis that neuroendocrine cells contain NSE, quantitation of NSE in rat, monkey and human tissues was accomplished by RIA. Previous work has demonstrated that NSE is a major soluble protein constituent of brain[12]. The levels of NSE in rat, monkey and human whole brain extracts range from 12,500 to 16,000 ng NSE per mg soluble protein (Table 1), representing 1 25–1.6% of the total soluble protein in brain.

Table 1 Neurone-specific enolase levels in various tissues of rat, rhesus monkey and man

Tissue	NSE (ng per mg protein) Rat	 Monkey	 Human	NSE Localisation by immunocytochemistry
Brain	12,500 ± 105	14,000	16,000	Neurones including central neuroendocrine cells of the hypothalamus
Pineal	2,650 ± 400	7,700	8,060	Pinealocytes
Adrenal	250 ± 11	—	—	
Cortex	—	50	100	None
Medulla	—	680	940	Chromaffin cells
Pituitary	2,000	—	600	
Anterior lobe	900	1,180	—	Anterior pituitary cells
Posterior lobe	3,400	2,870	—	Pars intermedia cells and endings of the hypothalamo–hypophysial system
Pancreas	18 ± 1.5	49	—	Islet cells
Thyroid	150	—	250	Presumed CT cells
Muscle	9	<1	—	None
Liver	4	1	3	None

Rat tissues such as pineal, adrenal, pituitary and thyroid represent pools of 5–20 animals. All tissue was homogenised in 20 mM Tris-PO_4, 2 mM $MgSO_4$, *p*H 7.4. The 100,000*g* supernatant was obtained. A double-antibody radioimmunoassay was developed for NSE from rat, monkey and human. Antiserum raised in rabbits to purified NSE-H was used in the monkey and human tissue assays and antiserum to purified NSE-R for the rat tissue. Each assay contained 4,000 d.p.m. of tritiated antigen (20-30 ng), prepared as previously described[12], antiserum (1:40,000 dilution for anti-NSE-R and 1:1,000 for anti-NSE-H) and the sample to be measured. The assay volume was 0.5 ml and all solutions were diluted in the assay buffer (0.15 M Tris-PO_4, *p*H 7.4, 2 mM $MgSO_4$ and 1% bovine serum albumin). The samples were incubated at 4 °C for 16 h followed by the addition of 250 μl of a 2 mg ml^{-1} solution of solid-phase anti-rabbit IgG (Bio-Rad Immunogarb). The second antibody incubation proceeded for 2 h at room temperature followed by washing and counting of the beads. Standard curves for each assay were linear over 2–100 ng. No cross-reactivity was observed with NNE (1 μg). S.e.m. are presented when four or more groups of animals were assayed.

Liver and muscle have very low levels of NSE that probably reflect the contribution of peripheral nerve processes present in these tissues. In contrast, significant amounts of NSE are present in the glandular tissues listed in Table 1, including levels in the pineal that approach those of brain. In contrast to pineal and pituitary, pancreas and thyroid have fewer APUD cells but still have levels of NSE that are clearly higher than those of liver and muscle. Assay of NSE in the adrenal medulla and cortex directly confirms the immunocytochemical localisation (Table 1). NSE levels by RIA are 10-fold higher in the medulla than in the cortex, consistent with the positive NSE staining of adrenal medullary chromaffin cells and non-staining of cortical cells.

The striking and consistent staining of APUD cells in rat and monkey by NSE antiserum coupled with quantitative evidence that in rat, monkey and man this represents actual NSE content makes NSE a unique, generalised molecular marker for these neurone-like cells. Whereas previous classification of APUD cells has had to rely on staining and physiological properties which occasionally proved difficult to document for a given cell class[8–10], the presence of NSE provides a simple and useful method for identifying members of this group. In cultured neuroblastoma cells (transformed cells of the APUD cell series), the levels of NSE correlate with the degree of differentiation induced by pharmacological manipulation[14]. NSE may, therefore, prove useful in the diagnosis and staging of tumours of APUD cells (apudomas) in humans.

The presence of a neuronal antigen in neuroendocrine cells further contributes to studies of the neurone-like character of these cells[10]. Not only do cells of the APUD classification have secretory properties similar to those of a neurone and, in most cases, a known associated peptide, they also exhibit excitable membrane properties in tissue culture[15]. As NSE has distinct

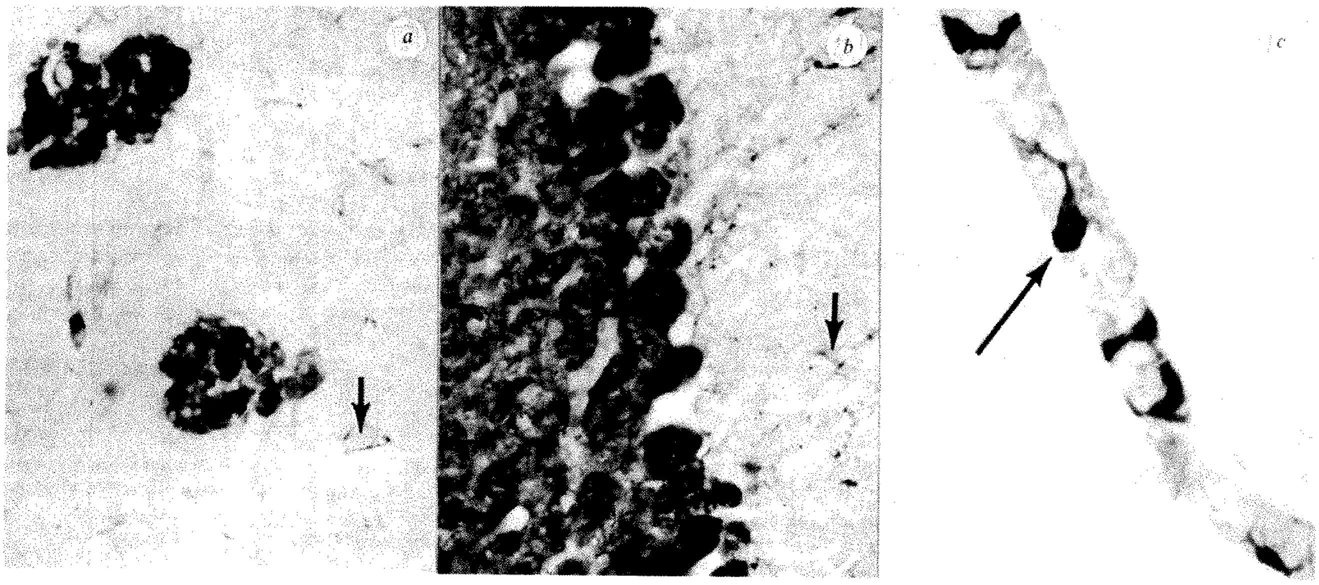

Fig. 1 Photomicrographs of rhesus monkey pancreas, adrenal and thyroid stained for NSE by the unlabelled antibody enzyme method. In each case, sections were incubated for 24 h with 1:1,000 anti-NSE antiserum. Controls carried out with preimmune and preabsorbed serum at 1:1,000 dilution showed no staining. *a*, 15-μm cryostat section of pancreas showing NSE staining of cells in islets of Langerhans. Some nerve processes are visible in the surrounding exocrine tissue (vertical arrow) but no epithelial or exocrine cells stain. ×130. *b*, 20-μm vibratome section of adrenal at the cortico–medullary border. Medullary chromaffin cells are stained and only occasional fragments of nerve processes stain in the cortex, but no cortical cells (vertical arrow). ×130. *c*, 15-μm cryostat section of thyroid showing the wall of one follicle with several NSE-positive cells (one is indicated by oblique arrow) amidst non-staining follicular cells. These positive cells are presumed CT cells. ×860.

structural and functional properties that may be an adaptation to the neuronal cytoplasm[7], the presence of NSE may be related to the neurone-like function of APUD cells. Alternatively, NSE may reflect the neural crest and neuroectodermal origin of these cells during development. Whatever the reasons for its presence, the availability of a generalised molecular marker for both peripheral and central neuroendocrine cells will be useful in studies of cellular differentiation both *in vivo* and in tissue culture. Finally, the possibility of simplifying and enlarging the APUD cell classification may settle some of the controversy concerning peptide-secreting tumours and their classification in man.

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Slow synaptic excitation mediated by serotonin in Auerbach's plexus

THE application of low concentrations of substance P to guinea-pig myenteric neurones has been reported by Katayama and North to produce depolarisation associated with an increase in input resistance and augmented excitability[1]. On this basis, they suggested that substance P may be involved in the production of the slow excitatory postsynaptic potential (e.p.s.p.) which can be evoked in myenteric neurones by electrical stimulation of the interganglionic connectives[2]. In this report, we show that application of serotonin (5-HT) mimics the slow e.p.s.p., and present several lines of evidence which suggest that 5-HT is a more likely candidate for the neurotransmitter of the slow e.p.s.p.

We used conventional methods to record intracellular electrical activity in myenteric ganglion cells of guinea-pig small intestine and to apply 5-HT iontophoretically to these cells[2]. Thirty-one ganglion cells were studied during electrode impalements which were maintained for 2–8 h. The effects of each drug and altered ionic composition of the bathing medium were always reversed by washing in normal Krebs solution, and all treatments were retested from two to four times on each cell.

The somal membranes of the 31 cells were relatively inexcitable in the absence of fibre tract stimulation and exogenous 5-HT. The cells would either not discharge in response to depolarising current pulses injected through the recording microelectrode or would fire only one, sometimes two, spikes only at the onset of the current pulse. When these spikes occurred, they were followed by prolonged (2–15 s) hyperpolarising after-potentials during which input resistance of the cells was decreased. These cells had resting potentials of −58 to −60 mV. Electrical stimulation of fibre tracts which entered the ganglia increased the excitability of the somal membranes of these cells. Increased excitability was reflected by maintained discharge of spikes that continued for 5–42 s after cessation of fibre tract stimulation (Fig. 1*a*), and by an increase in the number of spikes elicited by intracellular injection of a depolarising current pulse (Fig. 1*b*). In contrast to the relative inexcitability before fibre tract stimulation, immediately after and during such stimulation, spike discharge occurred throughout the application of depolarising pulses, and the frequency of spike discharge increased in direct proportion to the intensity of the injected current.

The increased excitability following fibre tract stimulation was associated with depolarisation of the cell membrane (−8 to −20 mV) and an increase in input resistance which persisted for 1–2 min after the end of the stimulation (Fig. 1*c*). During the excited state, the somal membranes discharged spikes at the offset of hyperpolarising current pulses, and these spikes as well as the continuous discharge were eliminated by hyperpolarisation of the membrane with steady hyperpolarising current injected through the recording micropipette (Fig. 1*c*). The increase in input resistance was not due to rectification because it still occurred when the depolarisation of the response was reversed by injection of hyperpolarising current.

The following observations indicated that the excitatory response to fibre tract stimulation resulted from release of a chemical substance. The response was (1) blocked in Krebs solution containing 16 mM Mg^{2+} and 1.25 mM Ca^{2+}; (2) blocked in HEPES buffered Krebs solution containing 1 mM

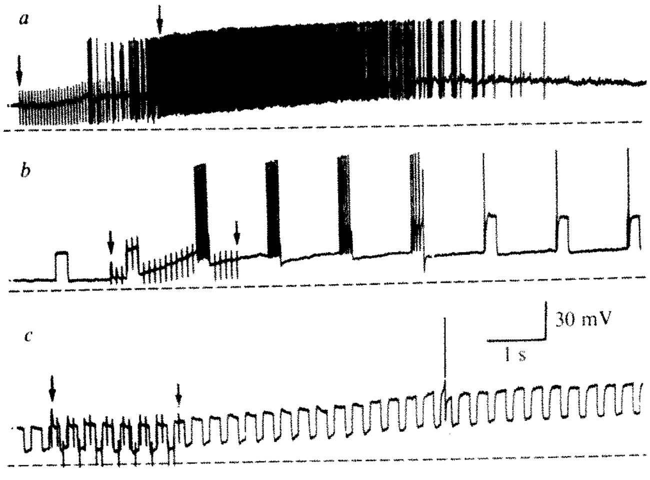

Fig. 1 Effects of fibre tract stimulation on guinea-pig myenteric neurones. *a*, Slow e.p.s.p. and spike discharge elicited by electrical stimulation of the interganglionic fibre tract (arrows indicate onset and offset of stimulus pulse train). *b*, electrical stimulation of fibre tract caused an increase in excitability as indicated by spike discharge throughout constant current depolarising pulses (0.4 nA). *c*, Fibre tract stimulation during repetitive injection of constant current hyperpolarising pulses (0.3 nA). Increased input resistance was reflected by increased amplitude of electrotonic potentials following fibre tract stimulation. Membrane potential was held 18 mV greater than resting potential by steady hyperpolarising current to prevent spikes at the offset of current pulses. One such spike still occurred.

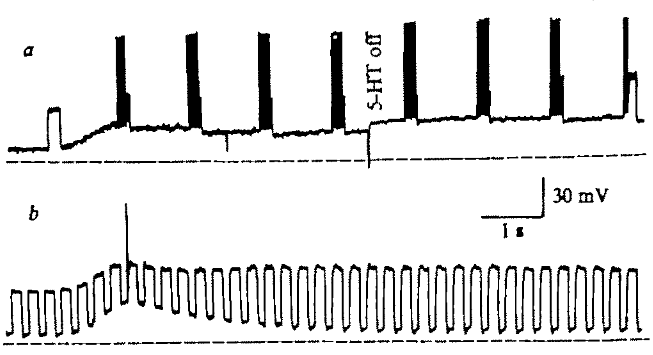

Fig. 2 Effects of microiontophoresis of 5-HT onto a guinea-pig myenteric neurone. *a*, Iontophoresis of 5-HT increased excitability and the cell discharged spikes throughout constant current depolarising pulses (4 5 nA). Iontophoretic current (300 nA) was turned on 9.5 s before the start of the record. Offset of the iontophoretic current is indicated. *b*, iontophoresis of 5-HT during repetitive injection of constant current hyperpolarising pulses (4.5 nA). Increased input resistance was reflected by increased amplitude of electrotonic potentials during 5-HT iontophoresis. The membrane potential was held 20 mV greater than the resting potential by steady hyperpolarising current to prevent spikes at the offset of current pulses. One such spike still occurred. Iontophoretic current was turned on 4.5 s before the start of the record and remained on throughout. Separation between the tip of iontophoretic pipette and the recording electrode was ~10 μm. The tip of the iontophoretic pipette did not penetrate the periganglionic sheath.

Mn^+; (3) enhanced in Krebs solution containing 7.5 mM Ca^{2+}. The response was unaffected by 6 μM nicotine, 10 μM prostigmine, 140 μM (+)tubocurarine and 140 μM atropine which suggested that the chemical substance was not acetylcholine.

Microiontophoretic application of 5-HT mimicked the excitatory response to fibre tract stimulation (Fig. 2*a*, *b*). When 5-HT was iontophoresed onto the cells, the membrane potential depolarised (–6 to –20 mV), input resistance increased, the cells discharged spikes throughout a depolarising current pulse, and frequency of spike discharge during a current pulse was directly related to the intensity of the depolarising current. The alterations of membrane potential and input resistance required 1–3 min to return to control levels after removal of the iontophoretic current. Iontophoretic current passed through control pipettes containing 3 M NaCl had no effect on the neurones. The 5-HT solution in the iontophoretic pipettes had a *p*H of 3–4; however, it is unlikely that the action of 5-HT was due to *p*H change because the 5-HT was ejected into a relatively large volume buffered at *p*H 7.4.

The excitatory responses to both fibre tract stimulation and exogenous 5-HT were reversibly blocked by 30 μM methysergide, a 5-HT antagonist. The responses to both nerve stimulation and iontophoresed 5-HT were reversibly blocked after addition of 5-HT (1–2.5 μM) to the perfusion solution. The excitatory response to iontophoresed 5-HT was unaffected by the presence of 16 mM Mg^{2+} and 1 mM Mn^{2+} in the perfusion solution.

The slow synaptic potential in myenteric neurones is associated with reduction of postspike hyperpolarising potentials and a dramatic increase in the excitability of the somal membrane. Earlier workers referred to this population of myenteric neurones, which generates prolonged hyperpolarising after-potentials, as either type II cells[3] or AH cells[4], and suggested that they were sensory neurones, because no synaptic input could be demonstrated. This seems unlikely in view of the present findings.

The following observations by others have implicated 5-HT as a neurotransmitter within the myenteric plexus. (1) Some of the 5-HT effects on contractile activity of intestinal musculature *in vitro* do not occur after neural blockade with tetrodotoxin[5,6]. (2) Extracellular recording from myenteric neurones shows an increase in the rate of spike discharge in the presence of 5-HT[7]. (3) Application of 5-HT increases the rate of release of acetylcholine from myenteric neurones[8]. (4) Serotonin, tryptophan hydroxylase and serotonin binding protein are present within myenteric neurones[9–12]. (5) Myenteric neurones synthesise 5-HT from its precursor, tryptophan[12]. (6) Tetrodotoxin-sensitive release of tritium-labelled 5-HT and its binding protein occur during transmural electrical stimulation of intestinal segments *in vitro*[13]. (7) Myenteric neurones possess a high-affinity uptake mechanism for 5-HT[14,15].

When the present results are considered with previous evidence, it becomes apparent that most of the criteria for establishing 5-HT as a neurotransmitter in Auerbach's plexus have been fulfilled: serotonin is synthesised and released by myenteric neurones; microiontophoresis of 5-HT mimics the action of the natural transmitter; actions of both the natural transmitter and 5-HT are blocked by the 5-HT antagonist, methysergide; tachyphylaxis occurs in excess 5-HT; inactivation mechanisms in the form of specific uptake of 5-HT exist in the myenteric plexus.

Our observations together with those of Katayama and North[1] suggest that a functional relationship between substance P and 5-HT may exist, because substance P is also present within the myenteric plexus. It is noteworthy in this respect that in the brain 5-HT and substance P co-exist within synaptic vesicles[16].

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Neurotransmitters decrease the calcium component of sensory neurone action potentials

RELEASE of neurotransmitters from presynaptic axon terminals requires the influx of Ca^{2+} ions during the nerve terminal action potential[1]. Action potentials recorded in some neurone cell bodies exhibit a relatively large Ca^{2+} component, and it has been suggested that these soma Ca^{2+} spikes may provide a model for Ca^{2+} influx across the less accessible nerve terminal membrane[2]. Recent data support the usefulness of this model. Serotonin (5-hydroxytryptamine, 5-HT) increases transmitter output at certain habituated sensory nerve-motoneurone synapses in the abdominal ganglion of *Aplysia* and it also prolongs the Ca^{2+} spike recorded in the sensory neurone cell body[3]. Enkephalin reduces the stimulated release

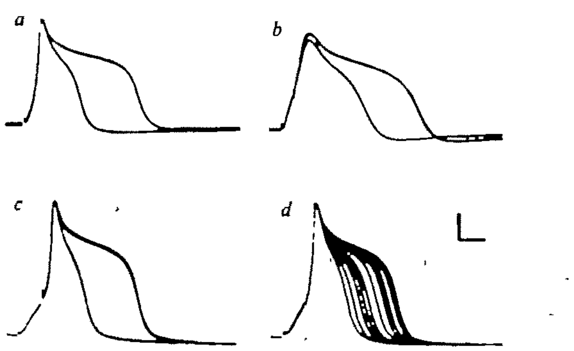


Fig. 1 The effect of *a*, 10^{-5} M 5-HT; *b*, 10^{-4} M GABA; *c,d*, 10^{-4} M NA on the DRG neurone soma action potential. The two successive sweeps show the spike before (longer duration) and after drug application Return to control duration shown in *d* with 10-s interval between sweeps. Calibration, 20 mV and 2 ms.

of substance P by adult cat trigeminal neurones[4] and by cultured embryonic chick dorsal root ganglion (DRG) neurones[5], and it decreases the quantal content of excitatory postsynaptic potentials (e.p.s.ps, transmitter unknown) evoked in cultured rat spinal cord neurones by co-cultured DRG cells[6]. This peptide also decreases the duration and magnitude of the Ca^{2+} component of the DRG soma spike[5]. With the thought that modulation of Ca^{2+} currents may be a general correlate of presynaptic inhibition, we have studied the effect of several putative neurotransmitters on the soma spike of cultured chick sensory neurones, and report here that they decrease the calcium component of cell body action potentials.

Lumbar and thoracic DRG were dissected from 9–11-d-old chick embryos, incubated for 20 min in 0.01% collagenase, mechanically dissociated into single cells by trituration through a Pasteur pipette, and plated in 35-mm collagen-coated Falcon tissue culture dishes. Cells were maintained in Eagle's minimum essential medium supplemented with 10% horse serum, 5% chick embryo extract, 2 mM glutamine, 22 mM glucose, 5 μg ml^{-1} streptomycin, 50 IU ml^{-1} penicillin and 1.0 μg ml^{-1} of 2.5S nerve growth factor (NGF) (purified from mouse salivary gland). Non-neuronal background cell growth was halted at confluence by γ-irradiation with ^{60}Co.

Using previously described techniques[7], intracellular recordings were made from DRG neurones which had been in culture for more than one week. The cells were bathed in a balanced salt solution containing 131 mM Na^+, 5.9 mM K^+, 5.4 mM Ca^{2+}, 0.8 mM Mg^{2+}, 0.1% glucose, 0.2% bovine serum albumin (BSA), and 25 mM HEPES, buffered to *p*H 7.4 with NaOH. The Ca^{2+} concentration was raised to 5.4 mM to increase inward Ca^{2+} currents and to improve the membrane seal around the microelectrodes. Known concentrations of drugs made up in recording medium were applied to individual neurones by pressure ejection from a 3–5-μm-tip micropipette positioned 100–200 μm from the impaled soma[8].

The action potentials of embryonic chick DRG neurones are composed of a fast Na^+ component blocked by tetrodotoxin (TTX) and a slower Ca^{2+} component that is in part responsible for the plateau during repolarisation[7]. γ-Aminobutyric acid (GABA), noradrenaline (NA), and 5-HT produced a reversible dose-dependent shortening of this plateau phase of the action potential (Fig. 1). In one series, 97% ($n = 36$) of the cells responded to GABA, 92% ($n = 52$) to NA and 83% ($n = 23$) to 5-HT. Dose–response curves for these drugs are shown in Fig. 2. The three drugs were found to exert their effects over approximately a 1,000-fold concentration range, but 5-HT and NA ($ED_{50} = 6 \times 10^{-7}$ M) were slightly more potent than GABA ($ED_{50} = 2 \times 10^{-6}$ M). With each drug, the maximum effect observed in control solution was a 60% reduction in spike duration measured at half amplitude.

Both the time course of drug action and the magnitude of the response were found to be dose related. Lower concentrations ($\sim 10^{-7}$–10^{-6} M) produced effects that lasted for several seconds, whereas return to control spike duration after application of higher drug concentrations (10^{-5}–10^{-4} M) took several minutes.

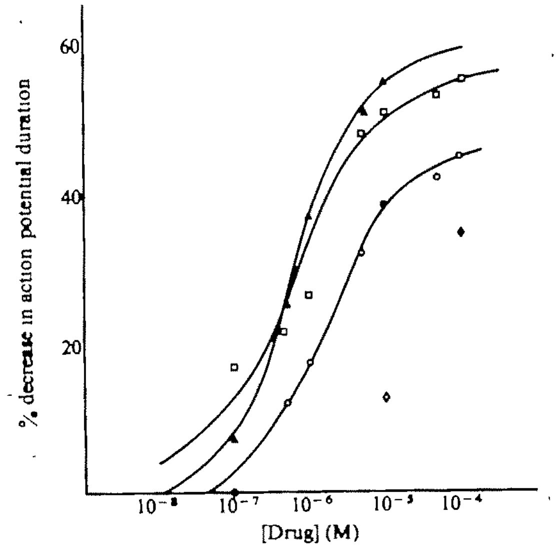
Glutamate produced a small and inconsistent effect on spike duration (Fig. 2). Only 8 of 17 cells responded to 10^{-5} or 10^{-4} M glutamate. ACh and glycine had no effect on the action potential in 10 neurones tested with 10^{-4} M. The fact that D-Ala-enkephalin amide and somatostatin at 10^{-6} M decreased DRG spike duration[5] was confirmed. Other peptides including substance P, bradykinin, neurotensin and thyrotropin-releasing hormone were ineffective at 10^{-5} M.

Resting membrane potentials (V_m) of the DRG neurones ranged between −36 and −64 mV, with a mean of -50.7 ± 0.7 mV (s.e.m., $n = 123$). Input resistance (R_m), measured as slope resistance through zero current, averaged ~50 MΩ. GABA, NA and 5-HT at concentrations of 10^{-7}–10^{-4} M did not alter these resting membrane parameters. A small decrease in R_m following application of 10^{-4} M GABA to DRG neurones has been observed within the first week after plating[8]. In these young cultures, the change in conductance was small, less than 4% of that observed in dissociated spinal cord neurones. No change in R_m was produced by GABA in the older cultures (> 1 week) used in this study. ACh at 10^{-4} M produced a 3–5 mV depolarisation associated with a small decrease in R_m.

We have begun to characterise the pharmacology of each response. The effect of 5×10^{-6} M GABA was not blocked by 10^{-4} M picrotoxin or 10^{-4} M bicuculline. These drugs are noncompetitive antagonists that block the GABA-mediated increase in Cl^- permeability at the crayfish neuromuscular junction[9] and in the vertebrate nervous system[10,11]. As no change in DRG resting membrane conductance was observed, it is not surprising that picrotoxin and bicuculline did not block the effect of GABA on the soma spike. Another kind of GABA receptor must be involved. The response to NA is apparently mediated by an α receptor: Selective α agonists, phenylephrine and dopamine at 10^{-5} M, were effective, whereas the β agonist, isoprenaline, at 10^{-5} M was not. Moreover, the effect of 10^{-5} M NA was blocked by 10^{-4} M phentolamine, an α antagonist, but not by 10^{-5} M propranolol, a β antagonist. Methysergide at 10^{-4} M did not block the effect of 10^{-5} M 5-HT.

The decrease in spike duration implies that the ultimate effect of GABA, NA and 5-HT is to decrease the duration of the inward Ca^{2+} current. It is not yet clear, however, if these drugs interact with Ca^{2+} channels directly. A decrease in spike duration might result from an increase in outward K^+ current.

Fig. 2 Dose–response curves for GABA (○), 5-HT (△) and NA (□) Responses to high concentrations of glutamate are included (◇). Each point is the average per cent in spike duration of a minimum of five neurones.



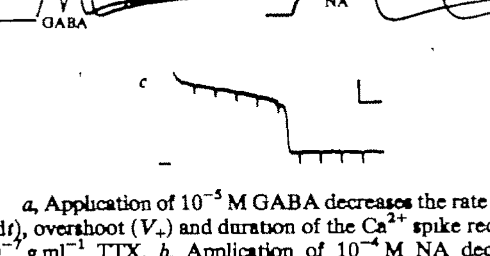


Fig. 3 *a*, Application of 10^{-5} M GABA decreases the rate of rise (dV/dt), overshoot (V_+) and duration of the Ca^{2+} spike recorded in 10^{-7} g ml^{-1} TTX. *b*, Application of 10^{-4} M NA decreases dV/dt, V_+ and duration of the Ba^{2+} spike recorded in TTX and 3 mM Ba^{2+}. *c*, Conductance during the plateau of a Ba^{2+} spike (5 mM Ba^{2+}) measured with hyperpolarising current pulses shows only a small increase in plateau conductance over rest. Calibration, 20 mV and 5 ms (*a*,*b*) and 100 ms (*c*).

Voltage-dependent and Ca-activated[12] K^+ currents must be considered. If this is the case, the outward current must occur early because GABA, NA and 5-HT also decrease the rate of rise and the peak amplitude of the Ca^{2+} spike (Fig. 3*a*). When the fast inward Na^+ current was blocked by 10^{-7} g ml^{-1} TTX, GABA (10^{-5} M) decreased the maximum rate of spike rise from 28 ± 2.6 V s^{-1} (mean ± s.e.m., $n = 10$) to 14 ± 2.5 V s^{-1} ($n = 10$) and the spike overshoot from 11.5 ± 1.1 mV (mean ± s.e.m., $n = 10$) to 3.0 ± 1.8 mV ($n = 10$). Moreover, the same drug effects were observed in the presence of 3 mM Ba^{2+} (Fig. 3*b*). (Ca^{2+} was held constant at 5.4 mM.) Ba^{2+} passes through activated Ca^{2+} channels[13] and blocks outward K^+ currents[14]. DRG spikes were prolonged in Ba^{2+} and the membrane conductance tested during the long plateau was low (Fig. 3*c*), indicating that K^+ channels were, in fact, blocked. Thus, considering the effect of the drugs on spike rate of rise and the persistence of the effect in the presence of Ba^{2+}, we favour a direct drug effect on Ca^{2+} channels. More direct evidence must be obtained by varying extracellular ion concentrations. In any case, our findings indicate that neurotransmitters may modulate voltage-sensitive neuronal membrane channels without producing a change in resting membrane parameters. Neurotransmitter modulation of voltage-sensitive channels has been described in studies of cardiac[15–17] and smooth[18] muscle.

Frank and Fuortes were the first to describe a type of inhibition of 1a e.p.s.ps that was not associated with a detectable change in the postsynaptic (motoneurone) membrane conductance[19]. The weight of evidence suggests that this 'remote' inhibition is primarily, if not entirely, due to inhibition of transmitter release from afferent nerve terminals[20,21] but the precise mechanism of presynatic inhibition remains obscure. Its temporal association with primary afferent depolarisation (PAD) has led to the notion that a transmitter-induced depolarisation of the primary afferent terminal reduces the size of the action potential and 1a transmitter output. GABA, the best studied neurotransmitter implicated in presynaptic inhibition, depolarises afferent nerve terminals (and nerve cell bodies)[11,22,23] by increasing the membrane permeability to Cl^- ions[11,23]. However, there is little direct evidence that this accounts for presynaptic inhibition.

Our results suggest an alternative mechanism. GABA decreases the influx of Ca^{2+} across the active soma membrane of cultured DRG neurones without producing a change in resting membrane conductance. If the same phenomenon occurs at nerve terminals this might effectively reduce transmitter release. It is interesting that this action of GABA is mimicked by 5-HT and NA because descending adrenergic (locus coeruleus)[24] and serotonergic (nucleus raphe magnus)[25] pathways have been described that terminate in the dorsal horn. Iontophoretically applied 5-HT and NA inhibit the firing of some neurones in superficial laminae of the dorsal horn[26]. Although the locus of this inhibition is not known, presynaptic actions of NA and 5-HT have been reported at other synapses[27,28]. It remains to be seen which mechanism, decreased Ca^{2+} conductance or primary afferent depolarisation, is responsible for presynaptic inhibition *in vivo*.

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Adenosine inhibits the accumulation of cyclic AMP in cultured brain cells

ADENOSINE stimulates the accumulation of cyclic AMP in some tissues and cell types, but has inhibitory or biphasic effects on the accumulation of cyclic AMP in other cells. Londos and Wolff[1] have attempted to explain these diverse effects by the existence of two adenosine reactive sites on adenylate cyclase. The first is the R-site, occupancy of which usually leads to activation on adenylate cyclase and which for activity requires integrity of the ribose ring. Much evidence suggests that this site is identical to an extracellular receptor for adenosine[2–6]. The second type is the P-site, which mediates inhibition of adenylate cyclase and requires integrity of the purine ring for activity. Preliminary evidence suggests that this site is only accessible from the interior of the cell[1,3]. However, we report here that the inhibition by adenosine of the increase in the intracellular level of cyclic AMP evoked by isoprenaline in cultured glioblasts is mediated by extracellular receptors.

Brain cells from perinatal mice were obtained by mechanical dissociation of brain tissue and cultured as described previously[7]. The cultures consist mainly of epitheloid astroblasts[8], which can be differentiated into cells exhibiting astrocyte-like appearance[9]. They are considered as models for glial cells[7,10,11], as they express presumptive glial markers[12–15]. The cultures were incubated for 10 min at 37 °C in the presence of the various additions before the concentration of intracellular cyclic AMP

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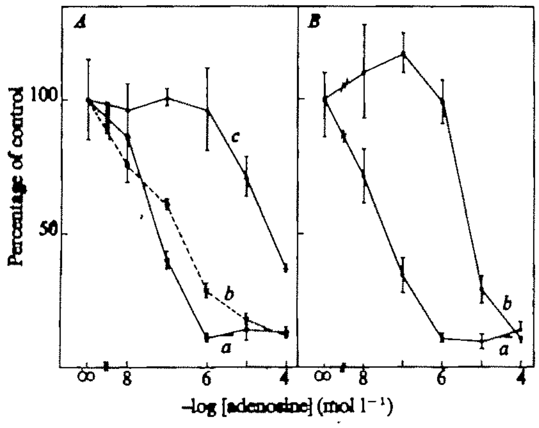

Fig. 1 Inhibition by adenosine of the elevation by isoprenaline (1 μM) of the level of cyclic AMP in mouse brain cell cultures in the absence (*A* and *B*, curve *a*) or presence of 0.5 mM Ro20-1724 (*A*, curve *b*), 0.5 mM MIBX (*A*, curve *c*) or, 0.5 mM theophylline (*B*, curve *b*). The culture for 24 d (*A*) or 20 d (*B*) of brain cells from perinatal mice (19–21 days *in utero*), the experimental incubation and the determination of cyclic AMP have been described elsewhere[7,16]. Protein content[28]:1.0 mg per plate. Controls (pmol cyclic AMP per mg protein): *A*: isoprenaline (1 μM), 580±90 (=100%); isoprenaline+Ro20-1724 (0.5 mM), 4,800±1,610 (=100%); isoprenaline+MIBX (0.5 mM), 4,700±290 (=100%); no addition, 7±2; adenosine: 1 μM, 8±2; 10 μM, 13±4; 100 μM, 16±3; Ro20-1724 (0.5 mM), 24±2, Ro20-1724 (0.5 mM)+adenosine: 1 μM, 22±4; 10 μM, 35±3; 100 μM, 73±13; MIBX (0.5 mM), 45±4 (duplicates); MIBX (0.5 mM)+adenosine: 10 μM, 44±2; 100 μM, 46±6. *B*: isoprenaline (1 μM), 480±70 (=100%); isoprenaline+theophylline (0.5 mM), 830±70 (=100%). All data given are mean values of triplicates ±s.d., except where indicated.

was determined[7,16]. Isoprenaline strongly elevates the level of cyclic AMP (ref. 7). This increase is inhibited by adenosine (Fig. 1*A* and *B*, curve *a*). Half maximal inhibition occurs at a concentration (IC_{50}) of 50–100 nM. The methylxanthines methylisobutylxanthine (MIBX) (Fig. 1*A*, curve *c*) and theophylline (Fig. 1*B*, curve *b*), known antagonists at adenosine receptors[2,3,5,17–20] and inhibitors of phosphodiesterase (PDE)[2,21], prevent this inhibition. The PDE-inhibitors 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro20-1724) (ref. 22) (Fig. 1*A*, curve *b*), dipyridamole and papaverine[2,21], however, alone or in combination (not shown) do not substantially influence the inhibition by adenosine. The blockage by methylxanthines of the effect of adenosine is therefore due to their potency as antagonists at adenosine receptors and is not related to inhibition of PDE. Dipyridamole is not only a PDE-inhibitor but also a strong blocker of adenosine uptake into cells[2,4,23]. In the primary cultures investigated here, 100 μM dipyridamole inhibited the uptake of adenosine (concentration range 0.1–10 μM) by 80 to 90% (data not shown). Ro20-1724 and papaverine have also been reported to inhibit the uptake of adenosine[4,23]. Neither dipyridamole nor a combination of 0.5 mM Ro20-1724, 0.5 mM papaverine and 0.1 mM dipyridamole significantly reduced the effect of adenosine (data not shown). Accordingly, the effect cannot be due to an intracellular action of adenosine but must be mediated by extracellular receptors.

Table 1 Potencies of derivatives of adenosine in inhibiting in cultured brain cells the increase in the level of cyclic AMP caused by isoprenaline

Compound	IC_{50} (μM)
N^6-(Phenylisopropyl)adenosine	0.005
2-Chloroadenosine	0.1
N^6-Methyladenosine	0.5
Adenosine N^1-oxide	0.5
3′-Deoxyadenosine	5
2′-Deoxyadenosine	10
7-Deazaadenosine	10
9-β-D-Arabinofuranosyladenine	50
2′, 3′-Isopropylideneadenosine	100

Experiments were performed with cells cultured for periods extending from 14 d to 23 d. The level of cyclic AMP measured after stimulation with isoprenaline (1 μM) varied between 300 and 900 pmol per mg protein; 0.8–1.0 mg protein per plate. Other details as in Fig. 1.

These receptors are different from the adrenergic α- and β-receptors present on the cells[7] because: (1) methylxanthines antagonise the effect of adenosine (Fig. 1*A*, curve *c*, Fig. 1*B*, curve *b*) but not of α- or β-adrenergic agonists[7,11]; (2) a 1,000-fold increase in the concentration of isoprenaline does not overcome the inhibition by adenosine[11]; (3) the α-adrenergic antagonist phentolamine (100 μM) does not alter the effect of adenosine[11]. Thus, the receptors mediating the effect described here are probably specific for adenosine or a closely related compound.

The potencies of a number of derivatives of adenosine are summarised in Table 1. These results indicate that alterations in the ribose moiety of adenosine abolish the inhibitory potency of the nucleoside, whereas substituents in the purine moiety are comparatively well tolerated. This is consistent with the proposed[1] requirements of the R-site of adenylate cyclase. Adenosine not only inhibits but also stimulates the accumulation of cyclic AMP in the cultured brain cells[11,24]. Also, this stimulatory effect is mediated by extracellular receptors which show the stereochemical requirements of an R-site. N^6-(Phenylisopropyl)adenosine, however, a compound which is at least ten times more potent than adenosine in eliciting the inhibitory effect (Table 1), is only slightly less potent than adenosine in stimulating the accumulation of cyclic AMP in the cultures (our work, in preparation). Thus, the simplest explanation for these two effects of adenosine is the existence of two different types of receptor for the nucleoside. We propose the name A1-receptor for the one that mediates the inhibition and A2 for the one that mediates the stimulation of cyclic AMP accumulation.

The content of cyclic AMP in the incubation medium (about 20% of total) changed in proportion with the intracellular level of cyclic AMP. Thus, neither an increased release of cyclic AMP nor an increased degradation by PDE (Fig. 1*A*, curve *b*) can be responsible for the decrease in the intracellular accumulation of cyclic AMP. Thus the effect is likely to be due to inhibition of adenylate cyclase. This inhibition is probably a direct result of the binding of the A1-agonist to its receptor. Extracellular humoral factors distinct from adenosine are unlikely to mediate the effect. This was shown in the following way. After exposure to cells for 10 min, incubation medium containing 1 μM isoprenaline and 1 μM adenosine was treated with adenosine deaminase (Boehringer) to destroy adenosine. Cultures incubated with this medium revealed the same content of cyclic AMP as those exposed to control medium (only isoprenaline present) (data not shown).

Adenosine increases by way of extracellular receptors, the level of cyclic AMP in brain slices[4,17] and in cultured cells of glial[2,11] or neuronal[19–21,23] origin. However, a receptor-mediated depression by adenosine of the level of cyclic AMP in brain slices has not been reported nor has it been described for any other system, with possibly one exception. In intact rat fat cells low concentrations of adenosine prevent the increase in the level of cyclic AMP that is evoked by noradrenaline[25,26]. This effect is not blocked by dipyridamole[26]. Whether or not it is antagonised by methylxanthines remains to be clarified[27]. Interestingly, N^6-(phenylisopropyl)adenosine is also more potent in this system than adenosine[26]. Glial precursor cells which constitute the majority of the cells in the cultures[7,8] are probably responsible for the effects described. The presence of A1-receptors on cultured brain cells encourages investigations into the possibility that A1-receptors are also present on mature glial or neuronal cells in the brain.

The results presented here imply[7,11] that A1-receptors and β-receptors are probably located on the same cells, most of which also display adrenergic α-receptors[7,11]. Such a combination of receptors may be used as an identity label during isolation of the cells from heterogeneous populations.

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Indomethacin in submicromolar concentrations inhibits cyclic AMP-dependent protein kinase

INDOMETHACIN is a potent inhibitor of prostaglandin synthesis[1,2], and has been used to demonstrate indirectly the mediation of biological events by prostaglandins. The rationale for this use of indomethacin has been supported when analytical techniques for direct prostaglandin quantitation have been used[3,4]. There are, however, conflicting data. A role for prostaglandins as the physiological messengers between hormones such as thyrotropin (TSH) and luteinising hormone (LH) and cyclic AMP has been proposed because prostaglandin antagonists inhibit the stimulatory effects of these hormones at their target tissues[5,6]. A similar role has been proposed in the action of cholera toxin[7,8]. In both cases, indomethacin failed to prevent the stimulatory effect of these agents on cyclic AMP production, giving support to the opposite view that prostaglandins were not essential intermediates[9–11]. Paradoxically, indomethacin suppresses, *in vivo*, thyroid hormone secretion[12] and cholera toxin-induced intestinal fluid accumulation[13–15]. A post-cyclic AMP site of action for indomethacin may explain these discrepancies. To elucidate the role of cyclic AMP-dependent protein kinase and endogenous protein phosphorylation in cholera toxin-induced intestinal secretion[16], we have tested the influence of indomethacin on partially purified protein kinase from rabbit ileal mucosa. We report here that indomethacin in submicromolar concentrations inhibits cyclic AMP-dependent protein kinase and endogenous protein phosphorylation.

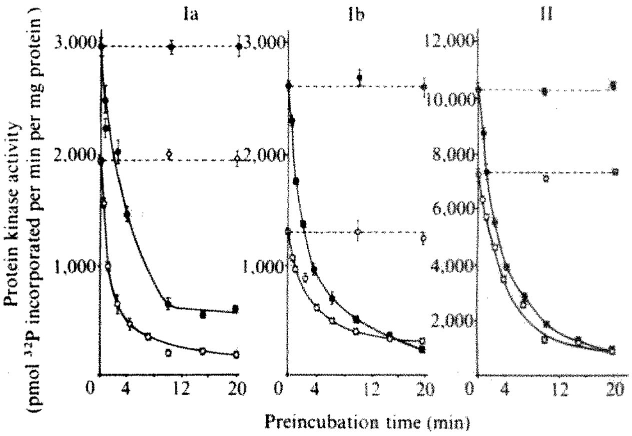

Fig. 1 Time-dependent blockade by indomethacin of types Ia, Ib and II cyclic AMP-dependent protein kinases. The standard assay mixture (final volume, 0.06 ml) for kinase activity contained: sodium α-glycerophosphate buffer (pH 7.5), 50 mM; dithiothreitol, 1 mM; protamine sulphate, 1.5 mg ml^{-1}; [γ-^{32}P]ATP, 6×10^{-4} M (10 μCi μmol^{-1}); magnesium chloride, 10 mM; enzyme protein, 0.02–0.2 mg ml^{-1}; ±cyclic AMP, 2×10^{-5} M and indomethacin, 1×10^{-7} M (solubilised by adding a minimum quantity of 0.1 M NaOH to the aqueous suspension of the drug). Enzyme protein was added to assay mixture containing all components except Mg^{2+}-^{32}P-ATP and preincubated for the indicated times at 30 °C with (●——●) and without (○——○) cyclic AMP. The reaction was started by the addition of Mg^{2+}-^{32}P-ATP. After incubation for 4 min at 30 °C, the reaction was ended by the addition of 1 ml of cold 10% trichloroacetic acid (TCA) followed by bovine serum albumin, 100 μg, as a carrier. The protein precipitate was collected on Whatman GF/C class fibre filters, washed 5 times with 1-ml portions of cold 10% TCA, transferred to 10 ml of a Triton toluene-based scintillant, and counted in a liquid scintillation spectrometer. Protein concentration was determined by the method of Lowry *et al.*[21] with bovine serum albumin as protein standard. Control values for protein kinase activity measured in the absence of indomethacin are depicted with (●----●) and without (○----○) cyclic AMP added. Each point represents the mean value ± s.e.m. obtained in 4 experiments carried out in a similar manner.

Homogenates of ileal mucosa were prepared by a modification of the method described previously[17]. The homogenation was carried out in 10 mM α-glycerophosphate buffer, pH 7.5, containing 1 mM dithiothrietol. A 150,000g cytosol fraction was prepared by the method of Uno *et al.*[18] and treated with solid ammonium sulphate followed by stirring for 30 min. Precipitation occurring between 30% and 55% saturation was removed by centrifugation at 20,000g for 20 min. The pellet was dissolved in and dialysed overnight against the α-glycerophosphate buffer. After dialysis the resulting solution was applied in a 2-ml volume to a column of DEAE-cellulose (1.5 × 60 cm) previously equilibrated with 10 mM α-glycerophosphate buffer (pH 7.5)/1 mM dithiothreitol. Protein kinases were eluted from

Table 1 Inhibition of rabbit ileal mucosa protein kinase activity and endogenous protein phosphorylation by indomethacin

	Protein kinase fractions					
	Ia		Ib		II	
Indomethacin effects	+Cyclic AMP	−Cyclic AMP	+Cyclic AMP	−Cyclic AMP	+Cyclic AMP	−Cyclic AMP
Inhibition (ID$_{50}$, M)						
Protein kinase activity	3.4×10^{-8}	1.7×10^{-8}	4.8×10^{-8}	1.7×10^{-8}	3.2×10^{-8}	1.8×10^{-8}
Endogenous protein phosphorylation	1.1×10^{-8}	0.60×10^{-8}	0.8×10^{-8}	0.62×10^{-8}	0.70×10^{-8}	0.58×10^{-8}
% Inhibition (1×10^{-7} M)	71 ± 2.1	84 ± 1.0	80 ± 1.2	88 ± 3.1	86 ± 1.0	87 ± 1.0

Values for % inhibition are mean ± s.e.m. of 4 determinations.

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the column with a linear gradient (0–0.8 M) of NaCl. Three major cyclic AMP-dependent kinase activity peaks were resolved and arbitrarily designated types Ia, Ib and II. The peaks of protein kinase activity were eluted at the following approximate concentrations (in M) of NaCl: type Ia, 0.16; type Ib, 0.33; type II, 0.51. The active fractions under each peak were pooled and concentrated by ultrafiltration.

Our results demonstrate that indomethacin at submicromolar concentrations can inhibit the three active forms of cyclic AMP-dependent protein kinase resolved from rabbit ileal mucosa in a time-dependent and concentration-dependent manner. Indomethacin had no instantaneous effect on the rate of ^{32}P incorporation into protein substrate. After 4 min, the incubation time of the standard assay for protein kinase, the per cent inhibition ±1 s.e.m. at 10^{-7} M indomethacin was (+cyclic AMP/−cyclic AMP) $17\pm3.7/20\pm4.3$ for peak Ia, $11\pm2.1/17\pm3.8$ for peak Ib and $13\pm2.9/16\pm6.2$ for peak II. Preincubation of the enzyme for different times before the addition of substrate led to progressive inhibition (Fig. 1). These results suggest that indomethacin takes part in a time-dependent reaction before it can produce its maximal inhibitory effect. The inhibition of cyclic AMP-dependent protein kinase activity by indomethacin was concentration-dependent (Fig. 2), and could not be reversed by dilution of the inhibitor, suggesting it was either irreversible or a result of tight binding by indomethacin (data not shown). Putative phosphorylation of endogenous protein substrate was measured by the incorporation of ^{32}P from ^{32}P-ATP to trichloroacetic acid-insoluble material (without exogenous protamine as substrate) of each activity peak. The inhibition response was qualitatively similar to the results obtained when exogenous substrate is added (Table 1).

The ID_{50} (concentration of inhibitor required to inhibit 50% of enzyme activity)[19] and per cent inhibition are shown in Table 1. The ID_{50} for indomethacin, when computed for protein kinase activity, ranged from 1.7×10^{-8} M (−cyclic AMP) to 4.8×10^{-8} M (+cyclic AMP). The ID_{50} for endogenous protein phosphorylation ranged from 0.58×10^{-8} M (−cyclic AMP) to 1.1×10^{-8} M (+cyclic AMP). The per cent inhibition for protein kinase activity at 1×10^{-7} M indomethacin varied from 71% to 88%.

In addition to inhibiting prostaglandin synthetase, indomethacin exerts effects on a variety of enzyme systems at micromolar concentrations or greater[3], properties which should be kept in mind when using this drug to evaluate the role of prostaglandins in biological events. On a molar basis indomethacin was between 79 and 224 times more potent as an inhibitor of cyclic AMP-dependent protein kinase activity than of prostaglandin synthetase activity (rabbit kidney microsomes[3]). Furthermore, at normal therapeutic doses, the free plasma concentration of indomethacin (5.6×10^{-7} M)[4] is 12–33 times greater than the ID_{50} for protein kinase.

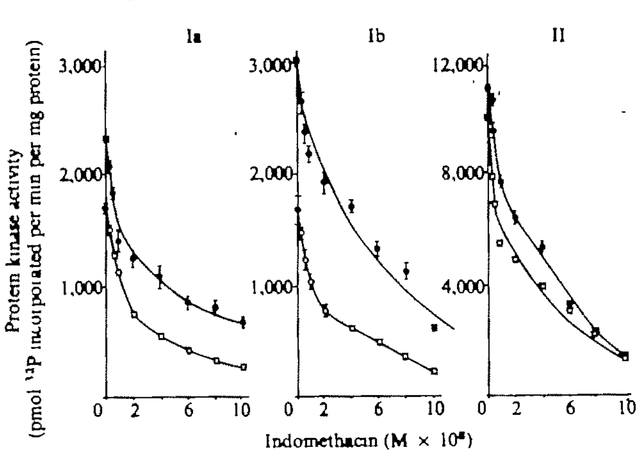

Fig. 2 Concentration-dependent inhibition by indomethacin of types Ia, Ib and II cyclic AMP-dependent protein kinase activities. Enzyme protein was added to the assay mixture containing the indicated concentration of indomethacin and preincubated for 20 min before starting the reaction by the addition of the substrate, Mg^{2+}-^{32}P-ATP. Each point, with (●——●) and without (○——○) cyclic AMP, represents the mean value ± s.e.m. obtained in 4 experiments carried out in a similar manner.

This study demonstrates that indomethacin is a potent inhibitor of cyclic AMP-dependent protein kinases and endogenous phosphorylation. It has been suggested that activation of protein kinase represents a major mechanism by which cyclic AMP carries out its function as an intracellular messenger in mammalian systems[20]. These data suggest that at therapeutic doses, indomethacin may function as an antagonist to cyclic AMP. Further studies are necessary to identify the mechanism of inhibition and to establish whether this new action of indomethacin occurs more generally in different tissues.

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Agropine—a major new plasmid-determined metabolite in crown gall tumours

THE crown gall tumour system may represent an elegant form of biochemical parasitism, in which the tumour-inducing bacterium *Agrobacterium tumefaciens* can, by insertion[1] and expression[2] of genetic sequences carried on the tumour-inducing (Ti) plasmid[3], determine the synthesis of unusual amino acids in transformed plant cells. These abnormal metabolities can be specifically utilised as nitrogen sources by the particular strain of inducing bacterium[4]. So far, two classes of plasmid-determined product have been recognised: the octopine family—octopine[5], octopinic acid[6], lysopine[7] and histopine[8]—and the nopaline family—nopaline[9] and nopalinic acid[10]. Whether the octopine or nopaline family of amino acids is synthesised by a particular tumour depends entirely on the type of Ti plasmid present in the inducing bacterium[11]. We report

Table 1 Octopine, nopaline and agropine degradation by *A. tumefaciens* strains and synthesis by crown gall tumours induced by those strains

Bacterial strain	Bacterial degradation*			Synthesis in tumour†		
	Octopine	Nopaline	Agropine	Octopine	Nopaline	Agropine
A6	+	−	+	+	−	+
A66	+	−	+	+	−	+
B6	+	−	+	+	−	+
181	−	+	−	−	−	−
38-9	−	+	−	−	+	−
T-37	−	+	−	−	+	−
C-58	−	+	−	−	+	−

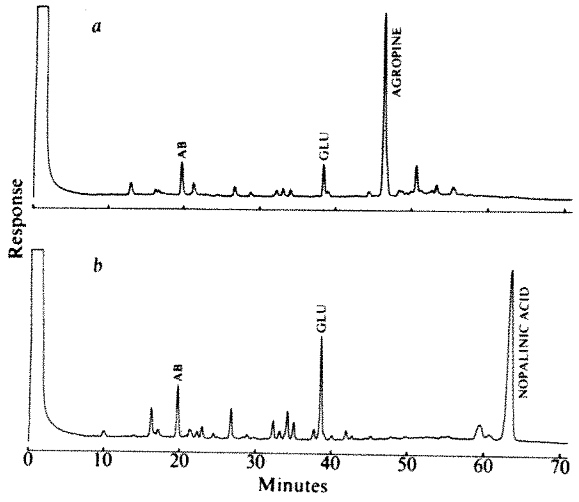
* Bacteria were inoculated into a liquid medium[16] containing either glucose (0.4% w/v) as C and energy source, and octopine (0.1% w/v) or nopaline (0.1% w/v) as sole N sources or agropine (0.1% w/v) as sole carbon and energy source, with ammonium sulphate (0.2% w/v) as N source. Agropine was isolated from an aqueous extract of A66-induced tumour tissue by adsorption on to Dowex 50×8, 20-50 mesh, H^+ form. The resin was washed with 10 bed volumes of water and eluted with 0.1 M ammonium hydroxide. Fractions equal to the column bed volume were collected, and those showing strong reaction with the alkaline silver nitrate reagent[13] after paper chromatography in propan-1-ol–ammonia (3:2 by volume) were combined and evaporated at 50 °C. When required, further purification was obtained by preparative paper chromatography. Cultures were shaken at 28 °C, and were examined for growth visually after 24 h. Disappearance of octopine or nopaline (measured colorimetrically using the α-naphthol-diacetyl reagent[17]) or disappearance of agropine (measured by gas chromatography[12]) correlated with visible bacterial growth.

† Synthesis of octopine, nopaline or agropine by bacteria-free tumour tissue was demonstrated by incubating the tumour tissue (~50 mg fresh weight) with either [U-^{14}C]arginine or [U-^{14}C]D-glucose (1 μCi, 304 μCi μmol^{-1}) for 24 h at 20 °C. The tissue was freeze dried and after grinding with sand, extracted with 2×5 ml water. After centrifugation the supernatant was passed through a column of Zerolit 225, 52-100 mesh, H^+ form, 1 ml bed volume. The resin was washed with water and cationic compounds (including octopine, nopaline and agropine) were eluted with 2M ammonium hydroxide. This eluate was concentrated by evaporation, and the individual compounds present were separated by two dimensional paper (Whatman 3 MM) chromatography (solvent I, 100 g phenol + 39 ml water; solvent II, butan-l-ol-propionic acid water 92:47:61), with added unlabelled octopine, nopaline and agropine. These compounds were located using either the Sakaguchi reaction[18] (octopine and nopaline) or the alkaline silver nitrate reagent[13], and the ^{14}C present in each compound was assayed by liquid scintillation. Radioactivity present in the agropine spot in '+' samples ranged from 1.7×10^4 c.p.m. (A66) to 3.4×10^4 c.p.m. (B6); '−' indicates c.p.m. $<10^2$. Lower limit of detectability was 10^2 c.p.m. No label was detected in agropine (added as a 'cold' standard before extraction) when healthy *N. tabacum* was incubated with ^{14}C-glucose as described above. R_f values for agropine were: solvent I 0.78; solvent II 0.25.

here the discovery of a completely new type of plasmid-determined compound which is present in prodigious amounts (up to 7% of the tumour dry weight) in tumours induced by octopine strains of *A. tumefaciens*.

Gas chromatograms of the heptafluorobutyric (HFB) *n*-propyl derivatives[12] of the free amino acids isolated from bacteria-free crown gall tumours initiated on 'White Burley' tobacco plants[10] by inoculation with any one of a series of octopine-metabolising strains of *A. tumefaciens* (A6, B6, A66) showed the presence of a major unidentified peak (Fig. 1*a*). This peak, which we have named 'agropine', was not detected either in tumours induced by nopaline-metabolising strains (Fig. 1*b*) of *A. tumefaciens* (C58, T-37, 38-9, 181) or in healthy tobacco tissue.

Fig. 1 Gas chromatograms of the HFB *n*-propyl derivatives[12] of the free amino acids extracted (see Table 1) from crown gall tumour tissue induced on *Nicotiana tabacum* by *A. tumefaciens* strain A66(*a*) and strain T37(*b*). AB, α-aminobutyric acid (added as internal standard); GLU, glutamic acid. Nopaline, although present in the T37 tumour at approximately the same level as nopalinic acid, does not chromatograph in these conditions.



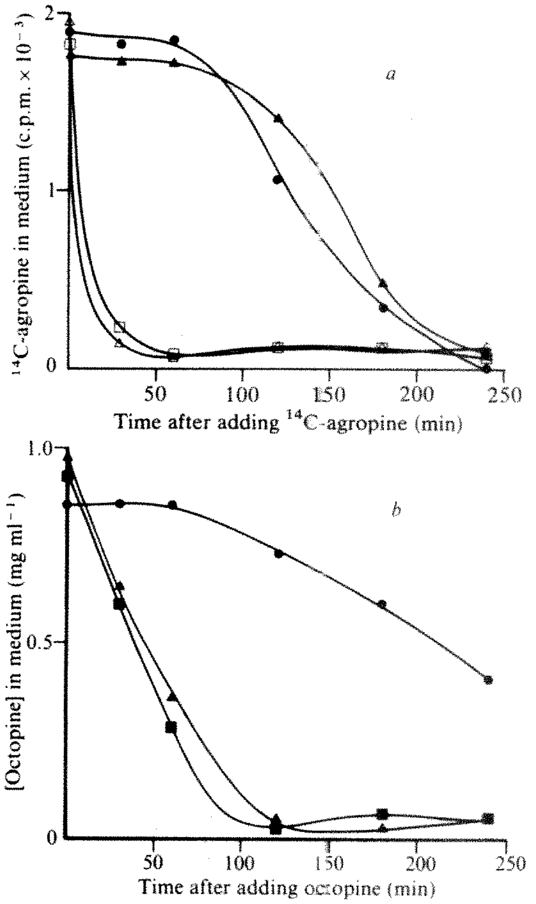


Fig. 2 Uptake of ^{14}C-agropine and octopine by *A. tumefaciens* strains. Bacteria were cultured at 28 °C in a liquid minimal medium[16] containing either glucose (0.4% w/v) + ammonium sulphate (0.2%), glucose (0.4%) + octopine (0.1%) or agropine (0.2%) + ammonium sulphate (0.2%). The bacteria were collected by centrifugation after 24 h, washed in minimal medium with no added carbon or nitrogen sources, and then resuspended in sufficient fresh minimal medium to give a final A_{630} value of 1.5. After addition of ^{14}C-agropine (~2×10^3 c.p.m., isolated from A66-induced tumour tissue after incubation with [U-^{14}C]glucose (see Table 1) or octopine (1 mg) the culture was shaken at 28 °C, and samples (10%) of the medium assayed, after centrifugation, by liquid scintillation (^{14}C-agropine uptake) or by the α-naphthol-diacetyl reagent[17] (octopine uptake). ●, Strain B6, not induced; ■, B6, induced with octopine; □, B6, induced with agropine; ▲, strain 1-1, not induced; △, 1-1, induced with agropine.

Transfer of the Ti plasmid from an octopine strain (B6S3) by conjugation in the presence of octopine (2 mg ml^{-1}), to a plasmid-cured rifampicin-resistant mutant of the nopaline strain C58, resulted in transconjugants (selected by plating on a minimal medium containing octopine (2 mg ml^{-1}) and rifampicin 25 μg ml^{-1}) which induced tumours containing agropine and octopine. These data suggest that agropine, like members of the octopine and nopaline families, is a strain-specific, plasmid-determined product.

The new compound was isolated (Table 1) from bacteria-free tumour tissue as colourless microcrystals from acetone–water, melting point 129 °C, $[\alpha]_D^{25}+19.5°$ ($c = 1$ in water). High resolution mass spectrometry indicated a molecular weight of 275.10254, corresponding to a molecular formula $C_{11}H_{17}NO_7$. Spectroscopic studies (IR, NMR) suggested a polyhydroxylic carboxylic acid. Gas chromatography of the HFB *n*-propyl derivative of purified agropine gave a single peak which co-chromatographed with the large peak found in the derivatised amino acid fraction from the tumour tissue. The molecule does not react with ninhydrin, but can be detected on paper using the alkaline silver nitrate reagent[13].

All octopine strains of *A. tumefaciens* (A6, B6, A66) examined so far utilise agropine as a sole carbon and energy source (Table 1). Uptake of agropine seems to be mediated by an inducible permease (Fig. 2*a*), and results obtained with the mutant octopine strain 1-1, which is constitutive for octopine uptake (Fig. 2*b*) and degradation, but not for agropine uptake (Fig. 2*a*) suggest that the uptake of octopine and agropine is controlled by different genes. Nopaline strains do not take up agropine after pregrowth in a minimal medium either with or without added octopine, nopaline or agropine, although these strains do transport and degrade octopine after pregrowth with nopaline.

The evidence suggests that agropine may represent a condensation product between a sugar or sugar derivative and an amino acid, and is therefore probably not synthesised by the enzyme responsible for synthesis of the octopine family of amino acids. Estimates of the amount of plasmid-derived genetic material in the tumour genome suggests that information sufficient to code for only four proteins of molecular weight ~50,000 is present[14]. Thus it is unlikely that agropine synthesis in the tumour could involve many intermediates not normally present in the plant tissue before transformation.

There is little doubt that agropine is not a normal plant product, although it is still conceivable that the capacity to synthesise agropine resides in the plant genome, and is only expressed in response to infection by a particular strain of infecting bacterium. However, this hypothesis is extremely unlikely since agropine is present in tumours induced on several unrelated genera of host plant[15].

In any event, the remarkably high levels of agropine present in tumours represent a major diversion of the plants' nutritional resources into a form which is specifically available as a carbon and energy source to the tumour-inducing pathogen. Agropine, together with the specific nitrogen sources octopine, octopinic acid, lysopine and histopine, could therefore provide the basic nutritional requirements of the pathogen in a form available only to that strain.

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A physically altered leucyl-tRNA synthetase complex in a CHO cell mutant

CONDITIONALLY lethal mutants in animal cells have been of great value in advancing our knowledge of genetics, biochemistry and molecular biology. Mammalian cells with temperature sensitive (*ts*) mutant phenotypes have been isolated by several laboratories, however no thorough characterisations of the altered gene products are available[1]. Selection procedures have been devised by Thompson *et al.* to isolate *ts* aminoacyl-tRNA synthetase variants in Chinese hamster ovary (CHO) cells which behave as missense mutants[2,3]. The CHO temperature-sensitive mutant *ts*H1 has very low *in vivo* levels of leucyl-tRNA, compared to wild-type levels at non-permissive temperatures; levels of the other 19 aminoacyl-tRNAs are unchanged[4]. This mutant also has an altered leucyl-tRNA synthetase which is thermosensitive *in vitro*[4], enzymatically different from wild-type leucyl-tRNA synthetase[5], and has an altered K_m for leucine[5]. We have now shown the leucyl-tRNA synthetase in *ts*H1 also has altered physical characteristics. The mutant enzyme exists primarily in a low molecular weight form whereas wild-type leucyl-tRNA synthetase is primarily associated with higher molecular weight particulate forms.

The particulate nature of aminoacyl-tRNA synthetases is a common feature of eukaryotic cells. The nature of these particulate enzyme forms reflects *in vivo* molecular organisation but its significance is not clear[6]. The size and form distributions of the 13 post-ribosomal particulate synthetases examined were indistinguishable in wild-type and mutant cells, except for leucyl-tRNA synthetase. The dramatic physical alteration of leucyl-tRNA synthetase is shown in Fig. 1. The distributions of

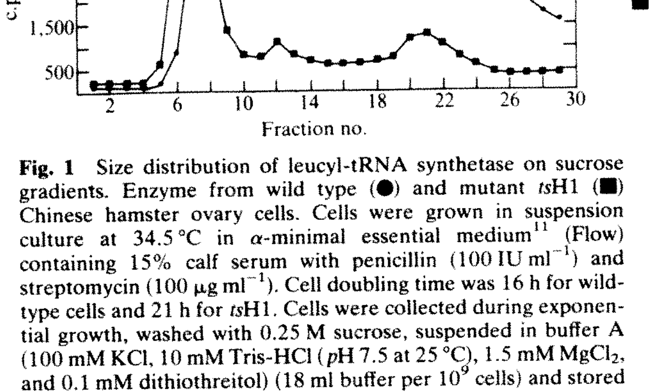

Fig. 1 Size distribution of leucyl-tRNA synthetase on sucrose gradients. Enzyme from wild type (●) and mutant *ts*H1 (■) Chinese hamster ovary cells. Cells were grown in suspension culture at 34.5 °C in α-minimal essential medium[11] (Flow) containing 15% calf serum with penicillin (100 IU ml^{-1}) and streptomycin (100 μg ml^{-1}). Cell doubling time was 16 h for wild-type cells and 21 h for *ts*H1. Cells were collected during exponential growth, washed with 0.25 M sucrose, suspended in buffer A (100 mM KCl, 10 mM Tris-HCl (*p*H 7.5 at 25 °C), 1.5 mM $MgCl_2$, and 0.1 mM dithiothreitol) (18 ml buffer per 10^9 cells) and stored at −90 °C. Cells (10^9) were broken in buffer A (20 ml) containing 1% Nonidet P-40 (Particle Data Labs) and supernatant was centrifuged at high speed to remove ribosomes. This postribosomal supernatant was centrifuged further to give a 6–40S subcellular pellet which was resuspended in buffer B (10 mM KCl, 10 mM Tris-HCl (*p*H 7.5 at 25 °C), 1.0 mM $MgCl_2$, and 0·1 mM dithiothreitol), layered on 10–30% w/v logarithmic sucrose gradients in buffer B, and centrifuged for 22 h at 25,000 r.p.m. in a Spinco SW-25.2 rotor at 4 °C (refs 12, 13). Sucrose gradients were assayed for the amino acids Arg, Asp, Cys, Gln, Glu, His, Ile, Leu, Lys, Met, Pro, Thr, and Val by esterifying ^{14}C-amino acids to cognate rat liver tRNAs and determining products formed (^{14}C-aminoacyl-tRNAs)[13,14].

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Pests of Grain Legumes: Ecology and Control

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February/March 1979, xvi + 454pp., £14.50/$30.00 0.12.646350.6

Grain legumes form an important part of the world's food crops especially in the tropics where they are the staple diet for many people. With the increase in research on these crops in recent years, it has become apparent that considerable loss in yield can occur as a result of insect pests. Entomologists working to try and reduce this problem, felt that more exchange of information was necessary and that a reference book specifically on grain legume entomology would be particularly welcome at this time. This volume, it is hoped, fulfils these requirements. It has its origins in an international symposium on pests of grain legumes, held at the International Institute for Tropical Agriculture, Ibadan, Nigeria.

Practical Aspects of Memory

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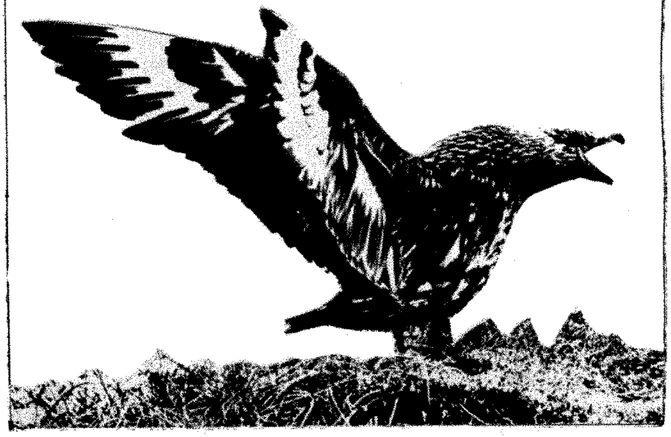

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the 12 indistinguishable activities are not shown here, as similar wild-type profiles have been previously published[6].

The wild-type leucyl-tRNA synthetase shows three particulate forms: an 8S form, a 20S form and a 30S form. Most of the wild-type enzyme activity is reproducibly found in the large 30S particle, and the mutant leucyl-tRNA synthetase is predominantly found in the small 8S form. The mutant leucyl-tRNA synthetase maintains negligible amounts of the higher molecular weight forms. These differences in synthetase forms provide additional evidence that the cause of the *ts*H1 phenotype is a directly altered leucyl-tRNA synthetase. These differences alone do not exclude the possibility that the shifts in leucyl-tRNA synthetase are due to a change in the molecules with which the enzyme is complexed, rather than a change in the leucyl-tRNA synthetase itself. However, previous studies on the mutant cells show this to be unlikely[4,5].

The physical evidence for a structurally altered leucyl-tRNA synthetase further supports the original evidence of Thompson *et al.*[4] that *ts*H1 is a true genotypic variant with an altered gene product and further counters epigenetic theories that somatic cell variants have stable phenotypes but do not contain changes in DNA structure. The electrophoretically altered hypoxanthine-guanine phosphoribosyltransferase from mutant HeLa cells[7-9] and cyclic AMP-dependent protein kinase from mutant S49 lymphoma cells[10] represent strong physical evidence for the existence of directly altered gene products and, consequently, stable genetic variants. The fact that the *ts*H1 gene product, leucyl-tRNA synthetase, is temperature-sensitive *in vitro*, and is apparently physically altered as well, suggests that it is also structurally altered due to a missense mutation in the structural gene.

The difference in synthetase forms between this mutant and wild-type cells is also very good evidence that the particulate nature of aminoacyl-tRNA synthetases is not an artefact but indeed a real intramolecular assemblage. The fact that large particulate forms of leucyl-tRNA synthetase are largely absent in the mutant although the mutant still survives suggests the complex is not completely essential for cellular function in the conditions described in this report. This mutant should prove very useful in determining the cellular role of the particulate forms of this enzyme.

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Nucleotide sequence at the insertion sites of a kanamycin transposon

THE TRANSPOSON Tn*903* carrying a gene for kanamycin resistance, is 3,100 base pairs in size and contains an inverted repeat sequence of 1,050 base pairs at both ends[1-4]. We report here the generation of a 9-base pair repeated sequence at the insertion site of Tn*903*. Tn*903* can be transposed to at least nine different sites on the coliphage fd DNA[1,2]. We have also transposed Tn*903* to three sites on small colicin E1 plasmid (ColE1) derivatives of about 1,600 base pairs. The presence of these many insertion sites on small recipient DNA molecules implies that no specific sequence is involved in the target sites on the recipient. To investigate the mechanism of transposition of Tn*903* at the molecular level, we have now analysed three independent insertions of Tn*903* on the small ColE1 DNA molecules. The nucleotide sequences of the three target sites and of the corresponding junctions between Tn*903* and the small ColE1 have been determined.

The small ColE1 derivatives used were pAO2 (ref. 5) and pAO3. pAO2 is constructed from the region between 0.75 and 1.00 map units of the ColE1 DNA molecule. (A map unit is the fractional length from the 5′ end of the H-strand[6] generated from ColE1 DNA by cleavage with the restriction endonuclease *Eco*RI.) pAO3 is similar to pAO2 but carries an additional region of 70 base pairs (Fig. 1). pAO43 (pAO2::Tn*903*), pAO48 (pAO2::Tn*903*) and pAO65 (pAO3::Tn*903*) were chimaeric plasmids carrying Tn*903*, constructed by transposing Tn*903* from R6-5 to either pAO2 or pAO3. These chimaeras contained a complete set of Tn*903*, demonstrated by their ability to donate the kanamycin marker to phage fd and λ*b5*. Restriction endonucleases used were *Hae*III, *Hap*II, *Hga*I, *Hha*I, *Bpa*I, *Bpa*II, *Hin*dIII and *Eco*RI. Methods for preparation of enzymes, plasmid DNA and restriction fragments have been described earlier[5,7]. DNA sequencing was carried out as reported by Maxam and Gilbert[8].

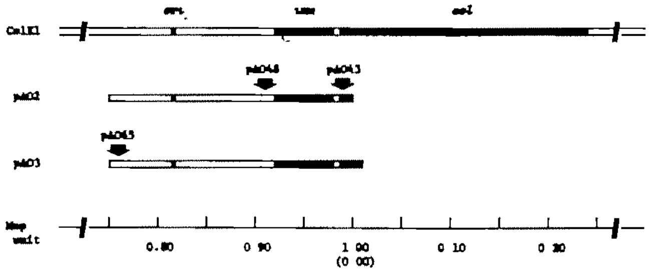

Fig. 1 Insertion sites of Tn*903* on the small ColE1 DNA molecules. The Tn*903* insertion sites in pAO43, pAO48 and pAO65 are shown by an arrow on the genetic map of pAO2 and pAO3. At the top is part of the genetic map of ColE1 as reference. DNA replication initiates at *ori* and proceeds towards the left. *col* is the structural gene of colicin E1 protein and *imm* is a gene conferring immunity to colicin E1 upon the host cell. The map position is indicated by the map unit of ColE1.

The Tn*903* insertion sites in pAO43, pAO48 and pAO65 were determined by comparison of restriction fragments produced from these plasmids with those from pAO2 or pAO3 by digestion with various restriction endonucleases (data not shown), and the sites were assigned to be 0.99, 0.91 and 0.76 map units on the small ColE1 DNA molecules, respectively (Fig. 1). The cleavage maps[5] around the three insertion sites and restriction fragments used for sequence analysis are shown in Fig. 2. The sequences within and surrounding the three insertion sites deduced are presented in Fig. 3*a–c*, in comparison with those in the corresponding region of pAO2 or pAO3. There are two particularly noteworthy features of the sequence. First, the two ends of Tn*903* are identical for at least 250 base pairs from the insertion site (Fig. 3*d*), in agreement with previous

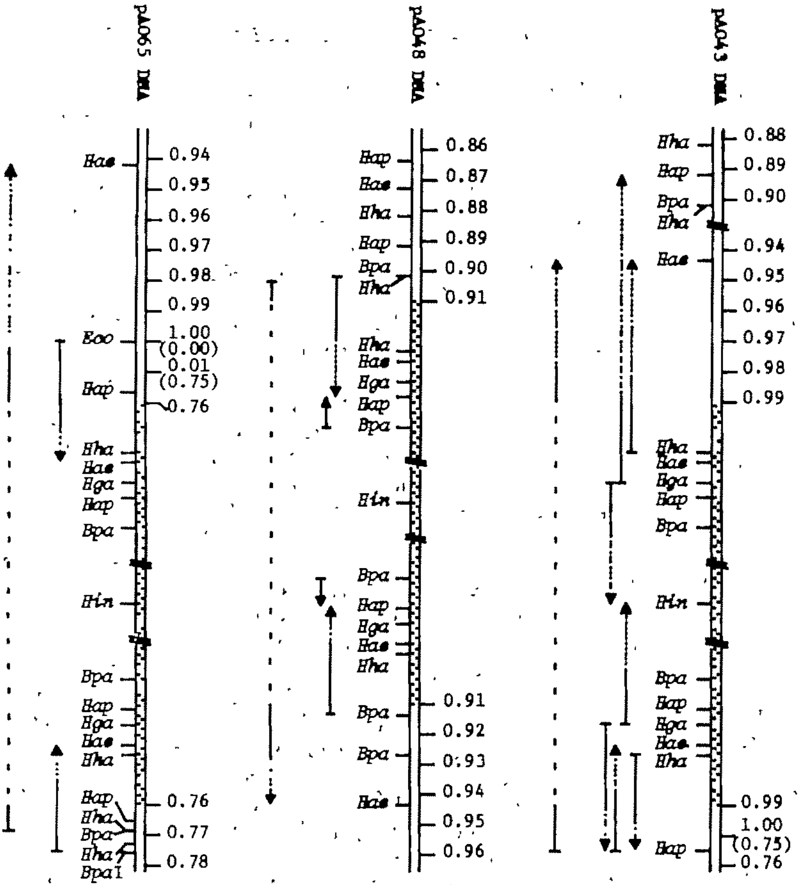

Fig. 2 Cleavage maps around the three insertion sites of Tn*903* and labelled strands used for sequence analysis. The map position is indicated by the map unit of ColE1 on each plasmid DNA. Cleavage sites by restriction endonucleases are shown under plasmid DNA: *Hae*, *Hap*, *Hha*, *Hga*, *Bpa*, *Hin* and *Eco* represent *Hae*III, *Hap*II, *Hha*I, *Bpa*II, *Hin*III and *Eco*RI, respectively. Arrows indicate [5'-^{32}P]labelled strands aligned in the 5' to 3' direction. The bottom arrow in each plasmid is a strand prepared from pAO2 or pAO3 DNA. All the strands were obtained by secondary cleavage of [5'-^{32}P]labelled double-stranded DNA. Solid lines in the arrows indicate the sequences that were resolved, and the dotted portions of the arrows indicate the sequences that were not as well resolved. The shading represents the Tn*903* DNA.

```
a                                   ▽▽▽▽▽▽▽▽▽
     pAO2   H 5' ATTTGGATTGCTAGGACTTTTTATATCAATAAAGAATAATTAAATCCCTAACACCTC
            L 3' TAAACCTAACGATCCTGAAAAAATATAGTTATTTCTTATTAATTTAGGGATTGTGGAG

                                            Tn903
                                    ▽▽▽▽▽▽▽▽▽_________________________▽▽▽▽▽▽▽▽▽
     pAO43  H 5' ATTTGGATTGCTAGGACTTTTTATATCAATAAAGGCTTTGTTG--/--CAACAAAGCCATCAATAAAGAATAATTAAATCCCTAACACCTC
            L 3' TAAACCTAACGATCCTGAAAAAATATAGTTATTTCCGAAACAAC--/--GTTGTTTCGGTAGTTATTTCTTATTAATTTAGGGATTGTGGAG

                                    ▽▽▽▽▽▽▽▽▽
b    pAO2   H 5' AAGGATCTCAAGAAGATCCTTTAATCTTTTCTACTGAACCGCGATCCCCGTCAGTTT
            L 3' TTCCTAGAGTTCTTCTAGGAAATTAGAAAAGATGACTTGGCGCTAGGGGCAGTCAAA

                                            Tn903
                                    ▽▽▽▽▽▽▽▽▽_________________________▽▽▽▽▽▽▽▽▽
     pAO48  H 5' AAGGATCTCAAGAAGATCCTTTAATCTTTTCTAGGCTTTGTTG--/--CAACAAAGCCTCTTTTCTACTGAACCGCGATCCCCGTCAGTTT
            L 3' TTCCTAGAGTTCTTCTAGGAAATTAGAAAAGATCCGAAACAAC--/--GTTGTTTCGGAGAAAAGATGACTTGGCGCTAGGGGCAGTCAAA

                                    ▽▽▽▽▽▽▽▽▽
c    pAO3   H 5' ACGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTGAGCGGATGCCGGGAG
            L 3' TGCTGTGTACGTCGAGGGCCTCTGCCAGTGTCGAACAGACACTCGCCTACGGCCCTC

                                            Tn903
                                    ▽▽▽▽▽▽▽▽▽_________________________▽▽▽▽▽▽▽▽▽
     pAO65  H 5' ACGACACATGCAGCTCCCGGAGACGGTCACAGCGGCTTTGTTG--/--CAACAAAGCCGGTCACAGCTTGTCTGTGAGCGGATGCCGGGAG
            L 3' TGCTGTGTACGTCGAGGGCCTCTGCCAGTGTCGCCGAAACAAC--/--GTTGTTTCGGCCAGTGTCGAACAGACACTCGCCTACGGCCCTC

d                       20                  40                  60                  80                 100
                         |                   |                   |                   |                   |
    5' GGCTTTGTTGAATAAATCAGATTTCGGGTAAGTCTCCCCCGTAGCGGGTTGTGTTTTCAGGCAATACGCACGCTTTCAGGCATACCTGCTTTCGTCATTT-
    3' CCGAAACAACTTATTTAGTCTAAAGCCCATTCAGAGGGGGCATCGCCCAACACAAAAGTCCGTTATGCGTGCGAAAGTCCGTATGGACGAAAGCAGTAAA-

                       120                 140                 160                 180                 200
                         |                   |                   |                   |                   |
       TGTTCAGCGCTCGTACCAGGGCCATAGCCTCCGCAACCTGACCATCGTAGTCACGCAGCGTCAGTGAACCCCCGAACAGCTGTTTTACCCGGTACATCGC-
       ACAAGTCGCGAGCATGGTCCCGGTATCGGAGGCGTTGGACTGGTAGCATCAGTGCGTCGCAGTCACTTGGGGGCTTGTCGACAAAATGGGCCATGTAGCG-
            HhaI          HaeIII                          HgaI                            HapII
                       220                 240
                         |                   |
       CGTTTCCGCTATCGAGCGACGGTTGTAATCTGTTGTCCATTTCCACCGCG---
       GCAAAGGCGATAGCTCGCTGCCAACATTAGACAACAGGTAAAGGTGGCGC---
                                                    BpaII
```

Fig. 3 The nucleotide sequence surrounding the Tn*903* insertion sites. The upper part in *a*, *b* and *c* represents the nucleotide sequence of the pAO2 or pAO3 DNA molecule, surrounding the Tn*903* insertion sites. The lower part in *a*, *b* and *c* shows the nucleotide sequence covering the junction between Tn*903* and either pAO2 or pAO3 in pAO43, pAO48 and pAO65, respectively. ▽ shows the duplication of 9 base pairs on Tn*903* insertion. *d*, The nucleotide sequence of the inverted repeat carried by Tn*903*. This sequence represents approximately 25% of the inverted repeat sequence. Nucleotide position is numbered from the integration site.

heteroduplex analyses[1,2,4]. This sequence has no obvious similarities to those of IS*1* and IS*2* (refs 9–12). Second, a 9-base pair segment, present at the site of insertion on the recipient DNA molecule, is repeated in the same orientation at both ends of Tn*903*. It is unlikely that the 9-base pair segment was brought from R6-5 as the donor of Tn*903* , because the sequence of 9-base pair repeat is different for each insertion analysed. Similar repetition has also been observed with insertion of IS*1* (refs 9, 10), Tn*3* (E. Ohtsubo, personal communication) and Tn*5* (H. Schaller, personal communication). Therefore, it seems that this duplication is a general feature of the Tn and IS translocation process, suggesting all Tn and IS use a similar mechanism of transposition.

The creation of a 9-base pair can be explained as follows. Two single-strand cuts separated by 9-base pairs are introduced on opposite strands of the recipient DNA molecule. The two new 5′ ends formed at the insertion site are then joined to the 3′ termini of Tn*903* so as to conserve the nine bases of the recipient DNA at both sides of the Tn sequence. The unpaired gaps at the complementary strands are then filled by repair synthesis. A similar explanation has been discussed in detail by Caros *et al.*[9] and Grindley[10].

Comparison of the three target sites indicated that neither the duplicated regions nor their vicinity contained an unique sequence common to three insertions. This suggests that no recognition of a specific sequence (recognition sequence) is involved for Tn*903* translocation, and that Tn*903* can be transposed to any site on DNA. However, we cannot rule out the possibility that the recognition sequence occurs very far from the target site for insertion.

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The chromophore binding space of opsin

THE rate of the reaction between opsin and 11-*cis* retinal, that gives the visual pigment rhodopsin, is measured in the presence and absence of structural analogues of 11-*cis* retinal, the polyene side chain of which is varied systematically. A decrease in regeneration rate can be interpreted as competition with 11-*cis* retinal for the binding site on the protein. This approach seems to provide information on the molecular dimensions of the chromophore binding space of opsin. Rotmans noticed that all-*trans* retinal does not inhibit the reaction between opsin and 11-*cis* retinal leading to rhodopsin[1]. More recently Mastumoto and Yoshizawa[2] have reported that β-ionone, a compound carrying the six-membered ring configuration of retinal but with a shorter side chain, does inhibit the rate of this reaction. The surprising discrepancy between these two observations has led us to study more systematically the effects on the regeneration rate of a number of 11-*cis* retinal analogues, in which either the polyene side chain or the aldehyde group has been modified. We report here results that suggest that opsin possesses a specific chromophore binding space, one dimension of which is determined by the longest axis of 11-*cis* retinal, measured from the hydrophobic binding site onwards.

Opsin, virtually free of rhodopsin, retinal and retinol, was prepared by sucrose density gradient centrifugation of bovine retina homogenate illuminated in the presence of NADPH (ref. 3). Aliquots were stored at −70 °C until used. 11-*cis* Retinal was isolated from a photoisomerate of retinal[4] (Eastman) and 11-*cis* retinol was prepared by $NaBH_4$ reduction of 11-*cis* retinal[5]. The purity of all compounds used was checked by spectrophotometry and thin-layer chromatography.

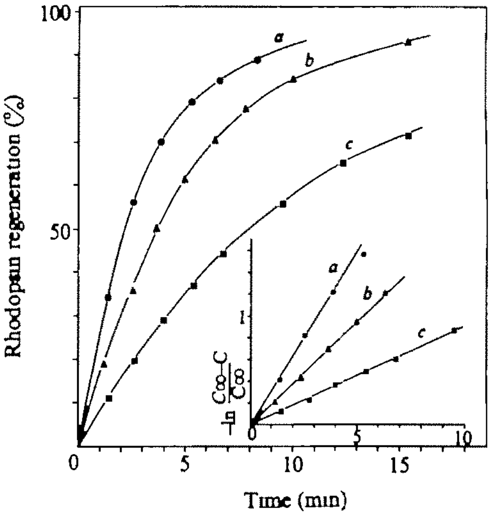

Fig. 1 Rate of the reaction between opsin and 11-*cis* retinal. *a*, In the absence of analogue; *b*, in the presence of the analogue 'C_{18}-ketone' (see Fig. 2*c*); *c*, in the presence of 'C_{15}-aldehyde' (see Fig. 2*b*). Reaction conditions, [opsin] = 9.5 μM; [11-*cis* retinal] = 48.5 μM, [analogue] = 500 μM; pH 6.5; 20 °C. Inset, semi-logarithmic plot.

The rate of the regeneration reaction was measured in a capped cuvette with two identical compartments. One compartment contained 600μl of the opsin suspension (19 μM in phosphate buffer) to which 10 μl of ethanol was added, either with or without the short chain analogue to be tested. The other compartment contained an equal volume of a suspension of 11-*cis* retinal (97μM in phosphate buffer, freshly prepared by adding the 11-*cis* retinal dissolved in 10 μl of ethanol). An initial absorption spectrum was recorded (Pye Unicam SP-1750, 20 °C), and the reaction started by mixing the contents of the two compartments. At various time intervals the absorption spectrum was recorded between 650 and 450 nm. The increase in absorbance at 520 nm (corrected for scattering differences at 650 nm) is used to measure the rate of rhodopsin synthesis[6]. Alternatively, aliquots were taken from the reaction mixture at various time intervals. The regeneration reaction was stopped by addition of NH_2OH (final concentration 100 mM), followed after 10 min by Triton X-100 (final concentration 1%); 500 nm absorbance was then measured before and after illumination. Both methods give essentially the same results. Difference spectra always indicate the formation of rhodopsin with λ_{max} 500 nm.

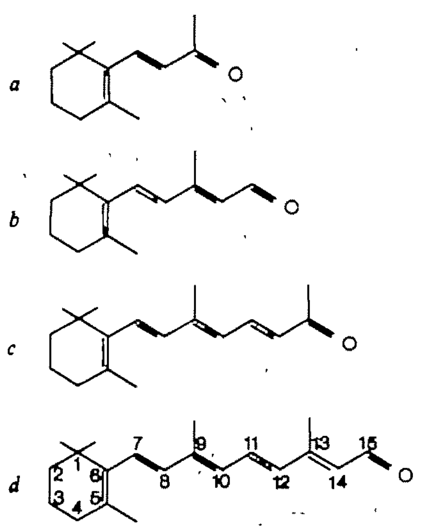

Fig. 2 Schematic structural formulae of short chain analogues of retinal. *a*, β-ionone; *b*, 'C$_{15}$-aldehyde', C$_{15}$H$_{22}$O, β-(3-methyl-5-oxo-1,3-all-*trans*-pentadienyl)2,6,6-trimethylcyclohexene; *c*, 'C$_{18}$-ketone', C$_{18}$H$_{26}$O, β-(3,7-dimethyl-7-oxo-1,3,5-all-*trans*-heptatrienyl)2,6,6-trimethylcyclohexene; *d* all-*trans* retinal.

Outer segment membrane suspensions rather than detergent solutions of opsin[2,6,7] have been used to approach the natural microenvironment of the protein as closely as possible. A fivefold molar excess of 11-*cis* retinal with respect to opsin was used. This results, in our conditions, in almost perfect first order kinetics over a large part (>65%) of the regeneration reaction, both in the presence and absence of analogues (Fig. 1). When 11-*cis* retinal is added to opsin as a very concentrated solution, a biphasic reaction is observed with a very rapid first phase. The analogues were preincubated for 10 min in the opsin suspension in a 10-fold molar excess with respect to 11-*cis* retinal. In this way reproducible results can be obtained.

Table 1 Apparent pseudo first order rate constants of the reaction opsin + 11-*cis* retinal → rhodopsin, in the presence and absence of various retinal analogues

Analogue	k_{app}(min^{-1})
No addition	0.33
β-Ionone	0.083
'C$_{15}$-aldehyde'	0.092
'C$_{15}$-alcohol'	0.084
'C$_{18}$-ketone'	0.20
All-*trans* retinol	0.19
11-*cis* retinol	0.18
All-*trans* retinal	0.32
13-*cis* retinal	0.36

Measurements of the regeneration rate of rhodopsin are presented in Fig. 1*a*. From the semilogarithmic plot (inset, *a*) a pseudo first order rate constant (k_{app}) of 0.33 min^{-1} was calculated. With regard to the effects of the analogues on the reaction rate, three patterns can be distinguished (Fig. 1, Table 1): (1) no effect is seen with all-*trans* as well as with 13-*cis* retinal (Fig. 1*a*), (2) moderate inhibition is observed with the 'C$_{18}$-ketone', all-*trans* retinol and 11-*cis* retinol (Fig. 1*b*), (3) strong inhibition is caused by β-ionone, the 'C$_{15}$-aldehyde' and the 'C$_{15}$-alcohol' (Fig. 1*c*). Varying the concentration of inhibitors between 0.25 and 5 mM shows that competitive inhibition occurs; the presence of even a large excess of analogues never prevents complete regeneration of rhodopsin.

An explanation of these observations originates from the conclusion of Matsumoto and Yoshizawa[2], that β-ionone competes with 11-*cis* retinal for a binding site on opsin. We observe that the 'C$_{15}$-aldehyde' inhibits the regeneration reaction to the same extent. As in the case of β-ionone[2], the presence of the aldehyde group is not essential, for the 'C$_{15}$-alcohol' gives essentially the same result. This seems to confirm the concept of a specific hydrophobic binding site for the trimethylcyclohexene ring on opsin. On extending the side chain of the analogues, the importance of a second parameter becomes apparent. The inhibition decreases markedly in the case of the 'C$_{18}$-ketone' and all-*trans* retinol. These two compounds contain a side chain with a rigid part consisting of four conjugated all-*trans* double bonds. It appears that the presence of a side chain, with a rigid part longer than that in the 11-*cis* retinal molecule, hinders the interaction of the six-membered ring moieties of these analogues with the hydrophobic binding site on opsin. This assumption seems to be supported by the fact that all-*trans* retinal, containing five conjugated all-*trans* double bonds in the side chain, does not inhibit the regeneration rate at all. Whereas the fairly high selectivity of retinal analogues with regard to the formation of visual pigments[8–11] suggests the existence of a rather specific chromophore binding space in rhodopsin, our findings indicate that such a specificity, with regard to the longest axis of the chromophore, is already present in the opsin molecule before the formation of visual pigment has taken place[12].

The effects of 11-*cis* retinol (moderate inhibition) and 13-*cis* retinal (no inhibition) are qualitatively compatible with our model. They may provide more refined information about the chromophore binding space of opsin, once their three-dimensional structure is known exactly.

In conclusion, competitive effects of retinal analogues with 11-*cis* retinal on the kinetics of the regeneration of rhodopsin from opsin provide information about the chromophore binding space of opsin. Apart from a previously noted[2] hydrophobic binding site for the six-membered ring of 11-*cis* retinal, it appears to be characterised by specific dimensions. The longest dimension of this space seems to coincide approximately with the long axis of the 11-*cis* retinal molecule.

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matters arising

Premelting near crystal defects

BARTIS[1] has suggested that the premelting increment in heat capacity, which has been observed for some materials to be of the form

$$\Delta C = A/(T_m - T)^2 \qquad (1)$$

may arise from melting below the intrinsic bulk melting point, in the vicinity of point defects. He also suggested that premature melting may occur in the vicinity of edge dislocations resulting in an incremental heat capacity of the form

$$\Delta C = B/(T_m - T)^3 \qquad (2)$$

The important question arises as to whether the constants A and B are large enough to account for the observed magnitude of the premelting heat capacity. We show that B is too small for the dislocation contribution to be significant, but that for lattice vacancies A is about as large as the observed magnitude.

Gronvold[2] records that 99.99% purity bismuth is 2% molten 3 K below the atmospheric pressure melting point. By using standard expressions for the excess pressure about a dislocation[3] one finds that a dislocation density in excess of 4×10^{14} lines m^{-2} is required to admit such a degree of premelting. Such a density is three to four orders of magnitude higher than the largest density one might expect for a stable dislocation array just below the melting point. For a more likely dislocation density at the melting point of 10^{10} lines m^{-2} bismuth would be 0.1% molten 0.07 K below its normal melting temperature which may be just within the limits of observation, but this may require a prohibitively high purity sample[2].

Neither, it seems, does the dislocation core melt at all. The problem of core melting of dislocations may be treated in the same manner as core sublimation as detailed by Frank[4]. He concluded that for all dislocations with Burgers' vector <1 nm the core is not hollow. This includes all simple metals and ionic crystals. Similarly, in the case of melting below the bulk melting temperature, it can be shown[5] that the melt radius for these crystals is always less than the core radius and therefore that core melting does not occur.

These ideas are borne out by experiment. Allnatt and Sime[6] have studied the premelting rise in the electrical conductivity of NaCl after 5% compressional deformation and concluded that no contribution to the premelting arises from the dislocations, and although the premelting surface conductivity is altered by deformation,[7] this is not due to dislocation core melting. Moreover, Amelinckx and Dekeyser[8], in reviewing the relevant experimental data, conclude that intrinsic melting does not measurably occur at grain boundaries.

It seems that premature melting near dislocations either does not occur or is so small as to be immeastırable. There may, however, be a possible source of premelting behaviour as described by equation (1), in the vicinity of vacancies. A vacancy in a metal usually results in a local decrease in pressure which, in the referenced case of bismuth, increases the local melting point, and this precludes vacancy-originated premelting. However, in the case of metals with normal melting curves the local melting temperature is depressed. A calculation of the magnitude of A in equation (1) reveals that the effect is sufficiently large to explain premelting over a degree or so below the melting point. This is illustrated by considering the depression of melting point that would occur if the total free energy of a system of vacancies were distributed uniformly through the crystal lattice. For a mole fraction α of vacancies with a Gibbs' function of formation of g_v each, in a crystal with a molar entropy of melting of ΔS_m the depression is approximately

$$\Delta T = \alpha N_A g_v / S_m, \qquad (3)$$

where N_A is Avogadro's number. For the following typical values for metals: $g_v \sim 1$ eV, $\Delta S_m \sim 8.5$ J mol^{-1} K^{-1} and α $(T = T_m) \sim 10^{-4}$ we find $\Delta T \sim 1$ K. There may therefore be observable premature melting in the vicinity of lattice vacancies.

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BARTIS REPLIES – I question Tallon's assessment of the stresses around edge dislocations. If the stresses are weak, then why are nucleation and growth alongside dislocations observed so often in structural studies of polymorphic transformations? Take, for instance, the passage of manganous oxide from the paramagnetic to the antiferromagnetic state. In neutron scattering from a single crystal above the Néel point Renninger *et al.*[1] detect peaks with satellites near the superlattice positions. They ascribe these features of the diffraction pattern to small domains of the magnetically ordered form of MnO. Using an electron microscope, Barber and Evans[2] do find small antiferromagnetic regions near dislocations and inclusions of Mn_3O_4 some 25 K above T_N. In the light of these results it is especially difficult to accept Tallon's claim that premelting does not occur in the cores of crystal dislocations.

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The Golgi complex and inter-relationships of arthropods

LOCKE AND HUIE have reported[1] that the Golgi complex beads in a wide range of arthropods, but not in the Onychophora or other soft-bodied invertebrates, have a common feature—they stain with bismuth. They suggest that this favours a monophyletic rather than a polyphyletic concept of arthopod evolution. It should be remembered, however, that the taxa listed with similar Golgi staining also depend on unstriated muscle (which facilitates extensive shape changes of the body), in contrast to the sclerite-bearing arthopods. The significance of the similarity between Golgi phenomena and unstriated muscle is obscure. However, it is well known that striated muscle would not suit the requirements of the Onychophora[2–6]. Also, it is certain that striated muscle has evolved more than once in the animal kingdom. The presence or absence of striated muscle in the Onychophora and in armoured arthropods seems to be of no more than functional significance.

Locke and Huie's opening paragraph does not seem to agree with present-day views on arthropod phylogeny. To take one point: by 1972 it was time to abandon the cumbersome phrase 'Onychophora–

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Myriapoda–Hexapoda assemblage' and to give a name, the Uniramia, to this group[7]. The component taxa are united on their basic arthropodan features; their particular type of jaws, and their limbs lacking any trace of true outer ramus. The jointed limbs of myriapods and hexapods could, arguably, have been derived from lobopodial limbs essentially resembling those of the extant Onychophora, and these limbs could have been derived from limbs of metamerically segmented worms with a haemocoel which, along with the muscles, worked the uniramous limbs. As long ago as 1940, Tiegs[8,9] emphasised the unity of these three taxa and Anderson[10] has shown in detail the common ontogenetic theme which exists throughout the Uniramia and contrasts absolutely with that pervading the entire crustacean group. Further, crustecean ontogeny could not have been derived from that of any known annelidan group. In contrast, the uniramian ontogeny might have been derived from that of some yolky-egged annelid or annelid-like worm which possessed a haemocoel and lobopodia, but not coelomate parapodia.

There is no justification on the basis of Golgi phenomena for the restriction of the term Uniramia to the Myriapoda and Hexapoda alone, nor for the exclusion from the arthropods of any animal on one character alone. That the Onychophora are thoroughly arthropodan is shown by their cuticle[11,12], which contains protein and chitin, but not collagen, in contrast to annelids; by the presence of an ecdysial cycle as in other arthropods[13], the cuticle being shed in one piece, as in no other invertebrates on Locke and Huie's list[1]; and by the general anatomy of the Onychophora, which tallies with that of other uniramian taxa. We do not know the significance of onychophoran Golgi phenomena nor of its association with unstriated muscle, but this one feature is no acceptable reason to disrupt the Uniramia or exclude the Onychophora from the arthropods.

Thus, without further consideration of the several other arthropodan taxa, it is clear that the Uniramia and Crustacea each represent well defined arthropodan taxa, which could not have originated from common annelidan ancestry, and that their cuticles must have evolved in parallel.

The exoskeleton of all arthropods has the same mechanical functions in providing unstretchable cuticles, sclerites and arthrodial membranes concerned with trunk movements and muscle insertions. The requirements of other invertebrates are different. The cuticles of the various arthropodan taxa are not identical, although protein and fine-fibre systems form the basis of most sclerites. There is thus no reason that the general manner of formation of arthropod cuticle may not have evolved more than once; nor is there any sound evidence against the unity of the Uniramia or against a polyphyletic concept of arthropodan evolution.

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Are viroids negative-strand viruses?

In their paper detailing the complete sequence of 359 bases in potato spindle tuber viroid (PSTV) RNA, Gross *et al.*[1] considered, as have other workers[2], that the circular single-stranded molecule is unlikely to function as a messenger RNA. The possibility that PSTV RNA might be a non-coding strand rather than a positive (coding) strand may have been overlooked.

I have examined the base sequence complementary to that established by Gross *et al.*[1]. Four possible initiation triplets (all GUG) and six possible strong termination triplets could result in four polypeptides containing 108, 79, 43 or 28 amino acids, of which the two larger (with molecular weights (MWs) 11,300 and 8,500) are the more interesting. The longest of these sequences begins with a GUG at positions 134–136 and teminates at a double UAG at positions 99–104 (Fig. 1). The GUG is preceded by a well-placed potential ribosome recognition sequence as indicated in Fig. 1. The second potential polypeptide is in a different reading frame and begins with a GUG at residues 177–179. It is preceded by an unbroken sequence of 14 purines, and teminates in an UAG at residues 55–57. The amino acid composition of the small polypeptide is unremarkable except for a high content of phenylalanine (10%), but the larger polypeptide has a content of arginine (11%) and lysine (5%) that would give it histone-like properties.

Two experimental observations would fit with the view that one or both of these polypeptides are produced by viroids. First, there is evidence for an RNA strand complementary to citrus exocortis viroid (CEV) RNA in infected tissues[3]; CEV is fairly closely related to PSTV[2]. Some of the hybridisable RNA was in the nuclear fraction but more was in the soluble cytoplasmic fraction, where it might have possessed a messenger function.

Second, an increase in amount (and in radioactive labelling) of two polypeptide bands in polyacrylamide gels was found in CEV-infected plants[4]. MWs assigned to the two polypeptides were 18,000 and 15,000. I have recalculated these MWs as 13,200 and 10,400, using as internal markers the large and small subunits of ribulose-1′,5′-diphosphatecarboxylase (MWs 54,000 and 12,000). The larger of the CEV-induced polypeptides was located preferentially in the histone fraction, the smaller was in the non-histone fraction[4]. This partitioning fits with the amino acid composition of the two polypeptide components that could be derived from the negative strand.

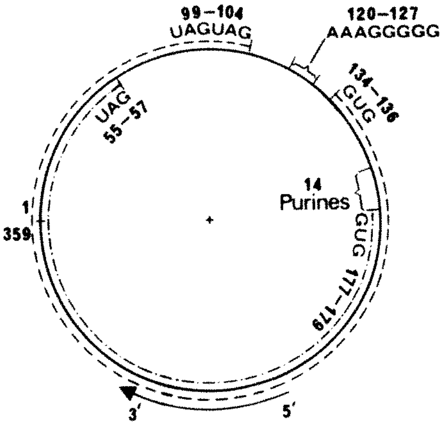

Fig. 1 Model of a PSTV RNA strand complementary to the viroid strand sequenced by Gross *et al.*[1]. The heavy line represents the circular RNA. The numbers 1 and 359 indicate the same start position as that used by Gross *et al.*, but because the strand represented here is antiparallel to the viroid strand, the position of base 1 in this diagram is equivalent to that of base 359 in the viroid sequence. Dashed line, polypeptide of MW 11,300; dotted-dashed line, polypeptide of MW 8,500.

In the light of the above evidence I suggest that viroids may be very small negative-strand viruses with the following properties. (1) They use secondary structure rather than a protein coat for protection of the genome; (2) they use a host polymerase to make a complementary RNA strand; and (3) this strand serves as a template for progeny viroid particles and as message for one or two viroid proteins. This hypothesis can be tested by a search in PSTV-infected tissues for one or two viroid-induced polypeptides of the predicted size and composition.

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reviews

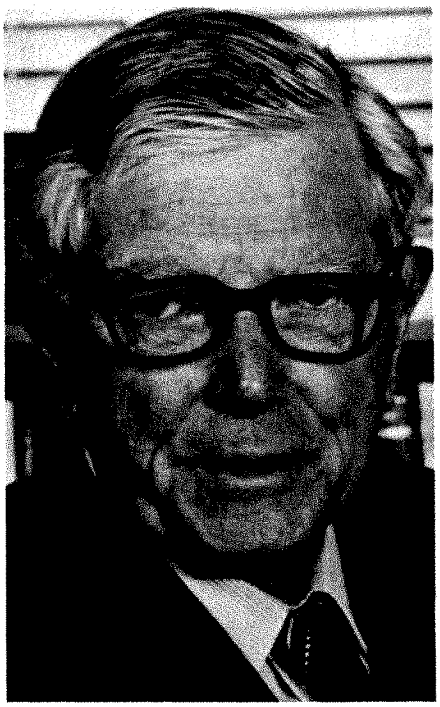
Thoughts on ageing

A. J. S. Davies

Endurance of Life: Implications of Genetics for Human Life. By Macfarlane Burnet. Pp. 230. (Cambridge University Press: Cambridge and London, 1978.) £8.95.

PERHAPS the best of this new book of Burnet's is the information it gives about the man himself and the relationship he has to the society in which he has become famous. Burnet states that one of the reasons that he writes is to try to clarify his own mind. In this instance the supposed topic for elucidation is ageing—not surprisingly somewhat of an obsession at the age of 78. In fact the book is a collection of essays, of variable quality, loosely related by their common authorship but little else. Aside from the need for self-expression Burnet also senses that as an eminent man his book may well be read by others, some of whom will be laymen. His concessions to the non-scientist are, however, by no means uniform. Diagrams of childlike simplicity are followed by pages of relatively unexplained and sometimes unrelated complexities, and I believe it highly unlikely that any but the most dedicated non-scientist will persevere with the book—which is a pity. Science is greatly in need of expositors of its meaning and basic facts.

Burnet's approach to human affairs is essentially that of a rationalist. He believes that there are facts of life which are not widely recognised, perhaps because of their unpalatability, but which nevertheless should be given wider public airing. As a geneticist Burnet of course espouses the basic assumption that that which is perceived of an organism, its phenotype, arises as a consequence of an interaction between the genetic information encoded in the DNA, the genotype, and the environment. He is unimpressed by those who suppose that whereas this is all right for sweet peas and field-mice it is a gross over-simplication for *Homo sapiens*. Burnet's stance in this old nature versus nurture argument is resolute. He believes that such human characteristics as intelligence and aggression must have a strong genetic component, despite the fervent hopes of educationalists and politicians alike that this is not so.


Sir Macfarlane Burnet

Contemporary society, according to Burnet, has a residue of activities, largely genetically determined, which were of advantage to the human species in the hunter-gatherer eras but for which the role has become less clear with the urbanisation and industrialisation of the past 10,000 years or so. A variety of social problems such as juvenile delinquency and the ennui of high rise flats (apartment blocks) seem to fall into perspective against such a back drop.

Statements of determinism have of course been made before, notably by Darlington. In Burnet they have engendered a massive pessimism arising perhaps from impotence at not knowing what to suggest if they are valid. Whatever the truth, the argument is a good one and is presented with a force which belies the years of the author.

Not content with a viewpoint which will antagonise almost every sociologist in the world, Burnet also points to the genetical homogenisation which he believes to be occurring in the middle echelons of society which could act to diminish human diversity to an undesirable degree. Reduction in family size he notes may prevent overpopulation but it will also disturb a natural balance which involved much natural selection within larger families. Burnet has also taken to heart the argument that one of the less desirable outcomes of modern medicine is the increasing preservation of the genetically unfit.

Burnet stops more or less short of the conclusions of the pre-war eugenicists but he nevertheless feels strongly that genetic counselling is worthwhile. He also believes severely handicapped children might well not be given the life support that they require.

He is an elitist who sees harm in differential breeding rates in the community which sometimes seem to favour the increase in the size of the populations of the under-privileged, but again he has no specific recommendations to make. His arguments are not in the main supported by an adequate bibliographical background but their emotional content is clear. Burnet sees himself as speaking with a common sense voice for the majority of intelligent men and women.

There are in the book many paradoxes. Burnet seems to equate power with evil on the basis of a definition of power as influence over people but he fails to realise that he himself is an extremely powerful and influential person but probably not evil. Goodness seems to be largely a feminine characteristic, a viewpoint which ought to make the more militant female liberationists fume.

The least satisfactory of the essays are those concerned with ageing. Briefly, Burnet sees ageing as due to the accumulation of genetic errors within somatic cells. Particularly important are errors in the error-rectifying system, which should have far more disastrous consequences than more mundane coding alterations. Burnet accepts all evidence for his theory but often seems disinterested in what scientists call the null hypothesis. He even has the face to criticise the virologists (p 39) for apparently adopting a similar attitude. Indeed Burnet's hostility to viruses borders on the irrational in that he seems unable to envisage that one of the genetic errors in somatic cells which he invokes so freely might in fact be the incorporation of a viral genome.

There is a whole chapter on *Xeroderma pigmentosum* which is seen, perhaps correctly, as a condition in which a deficient error repair mechanism leads to a variety of defects including a high susceptibility to ultraviolet light and increasing numbers of skin tumours. Burnet sees this unpleasant syndrome, which has a known genetic basis, as a grossly accelerated example of ageing corresponding exactly to his hypothesis. In addition the belief he has about cancers arising as a consequence of somatic mutations is confirmed. To one who has read a number of Burnet's previous books much of the early parts of the present volume may evoke a strong sense of *déjà vu.*

As, with succeeding chapters, Burnet moves from Ageing in Mammals by way of the Genetics of Power to his Vision of a Million Years the book becomes more readable. Those who wish to dip into it should in my opinion start at the back and work forward until they come to familiar ground. The most evocative and fluent passages are those which concern Burnet himself. He talks wistfully of societies in which privileged elders can retain an honoured position as men of experience and transmitters of traditional wisdom. Despite or perhaps because of this he makes statements of astonishing lack of insight—for example, "a little thought will convince most people that natural death means a death in which the essential factors concerned were genetic ones" The value judgment involved here in the use of the adjective essential and the apparent lack of realisation of the operation of the many stochastic events which could influence the matter are surprising.

Whenever he himself has not thought about a particular matter it is dismissed in a lordly manner—for example, "we need not worry too hard to imagine by what mechanism it ('psychosomatic' death may be achieved."

Few aspects of our contemporary society escape Burnet's pessimistic pen. Most of his views will be unpopular and one cannot help but agree with his statement that he has never failed to write, or modify what he has written, out of fear of the consequences. The book is erratic, aggravating, pontifical on occasions, sometimes clumsy, often obscure. but rarely dull It should be read and discussed ☐

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Atomic and molecular physics

Atoms and Molecules. By M. Weissbluth Pp.713. (Academic: New York, San Francisco and London, 1978.) $59.50; £38.65.

GROWN out of a full year graduate lecture course in atomic and molecular physics, this monograph is both a textbook and a reference manual of ambitiously wide scope. Thus the final chapter (28) on ligand fields treats such topics as electronic d^2 in a cubic field with spin-orbit coupling. And the chapter's last section concludes with a diagram that shows the energy levels of molecular orbitals when an octahedral complex AB_6 is involved; from a preceding table follows, for each symmetry associated with the levels, the structure of symmetry adapted orbitals for both the orbitals of the central atom A and the ligands B. It would be only a short step to frontier problems such as ferric ions in proteins. Derivations often take just a few lines—try this conclusively without simultaneously applying group theory to the central electrons and the lattice-like structure around them! All the required atomic and algebraic tools and data are lucidly expanded in the book, and referencing to them is done efficiently.

Naturally group theoretical topics take up most of the space in the first of the text's six parts, which gives the mathematical background. Only rigorous pruning to the needs of atomic and molecular physics, and resorting to summaries of selected properties where appropriate, keeps this part in size and readable. He who struggled through the thicket of the full representation theory of the permutation group, this centrepiece in problems involving identical particles, will appreciate the refreshing brevity and swiftness with which the relevant steps in Young's scheme evolve.

Part II, on the quantum-mechanical background, assumes some undergraduate course knowledge. The remaining four parts move on towards molecules: one-electron atoms (from Dirac's equation by way of Schrödinger's to hyperfine interactions), N-electron atoms, electromagnetic interactions (culminating in the Kramers-Heisenberg formula and in applications), and finally 130 pages on molecules, covering in particular the spectra of polyatomic molecules The notion of conjugate configuration, in the context of N-electron problems, tells immediately why no spin-orbit splitting occurs for half-filled shells. However, even then the mutual effect between electrons does give rise to some splitting. Such splitting may be ignored because of its smallness, though the author, in this rare instance, misses the cause when relating it to interaction solely between terms. All except purists are likely to overlook an unnecessary impediment to more fundamental approaches in some basic relationships: to equivalence a unit vector directly with its cartesian representation as a column matrix, and on occasion a state vector with the complete function—something quite commonly found in textbooks Despite these shortcomings the whole presentation is elegant and informative.

This new book shares one weakness with many a first edition: the index doesn't measure up to the text. Similarly, the references haven't quite outgrown a list for the students of recommended reading. There is one particular omission: among the three classics on group theory and quantum mechanics, which came out within a very short period around 1931, H. Weyl's and E. Wigner's books are mentioned, whereas B. L. van der Waerden's is not.

Who is going to buy the book? Sadly enough the price tag will turn away most graduate and postgraduate students. Needless to say that the book is well produced and the typographical layout excellent. But perhaps one should draw attention to over 70 useful tables, an impressive collection of information which, leaving aside the newly compiled tables, one might as well find scattered across several book-shelves.

Werner Elssner

Werner Elssner is Temporary Senior Lecturer in the Department of Physics and Astronomy at University College, London, UK

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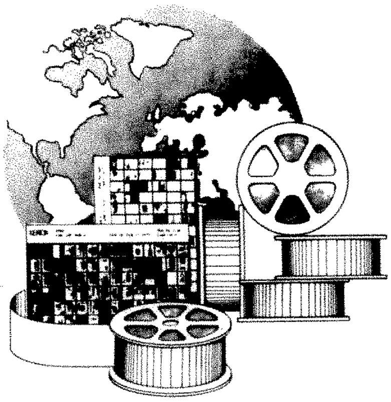

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LA TROBE UNIVERSITY
Melbourne, Australia
School of Biological Sciences
DEPARTMENT OF BIOCHEMISTRY

RESEARCH FELLOW

The appointee will participate in a project on "The biosynthesis of polysaccharides and polysaccharide-proteins in higher plants" with the chief investigators, Professor B. A. Stone and Dr G. B. Fincher. This project is funded by the Australian Research Grants Committee.

The Research Fellow is expected to have considerable experience in membrane isolation, fractionation and characterisation. Further experience in the areas of polysaccharide, glycoprotein or proteoglycan biosynthesis or characterisation would be advantageous but not mandatory.

The position will be for one year initially, although possible extension to two or three years may be approved after appointment. A travel and removal allowance will be paid.

Qualifications: A Ph.D. in Biochemistry or a cognate discipline.

Salary: A starting salary of $A15,179 per annum, with a range of $15,179 to $16,540 if the appointment is extended over three years.

Commencement date: As soon as possible.

Further information and application forms are available from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF. Applications close on **January 27, 1979.** 8919(A)

HARVARD SCHOOL OF PUBLIC HEALTH

The Harvard School of Public Health welcomes applications for a tenured Professorship in the biomedical sciences in the Department of Nutrition. The candidate is expected to head the Department, which has post-doctoral, doctoral, and Master's students, substantial laboratory space and some endowment. The University is committed to integrate the activities of its various faculties in both research and teaching, and this senior level person would be expected to forward these goals in the nutritional sciences, particularly as they relate to public health problems. Broad qualifications for the position might typically include a Ph.D. and/or M.D. degree; a distinguished record of biomedical research relevant to or involving nutritional sciences; ability to teach nutrition-related graduate courses, and to attract promising students and junior faculty to the field; leadership, administrative experience, and interdisciplinary orientation which includes an interest in health policy issues.

Send curriculum vitae, a list of publications, a statement of current and future interests in nutritional sciences, and the names of two individuals who would be willing to respond to a request for evaluation to:

Mrs. Emily Morrison
Harvard School of Public Health
677 Huntington Avenue, Room 1013.
Boston, Massachusetts 02115 U.S.A.

Harvard University is an equal opportunity employer with an affirmative action program. 8892(A)

COMMONWEALTH MYCOLOGICAL INSTITUTE
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Vacancies for

FOREST MYCOLOGIST and PLANT PATHOLOGIST

THE FOREST MYCOLOGIST will work on the fungal pathogens of Central American pines, in a post funded by the UK Ministry of Overseas Development, for a period of three years.

The successful candidate will be based at the Commonwealth Mycological Institute, but will be required to make annual collecting visits to Central America to survey and collect fungi associated with pines.

Qualifications: A good honours degree with postgraduate experience involving work on the identification of fungi. Experience in forest pathology will be an advantage.

THE PLANT PATHOLOGIST will work as a Liaison Officer under the UK Ministry of Overseas Development, based at the Commonwealth Mycological Institute.

He will assist developing countries on behalf of ODM with the identification of pathogens of forest trees; advise on control methods and supply information of current interest on forest pathology; conduct short-term surveys and make advisory visits overseas. He would be expected to contribute to the work of the Commonwealth Mycological Institute.

The post forms part of a small team comprising two Liaison Officers and two ancillary staff.

Qualifications: Preference will be given to a forest pathologist with knowledge of plantation problems. Tropical experience is essential.

Salary, FOR BOTH POSTS, in the scale £2,839 to £8,461 (which is subject to revision). Starting salary according to age, qualifications and experience.

Application forms, and further particulars, obtainable from: The Executive Director, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN.

Closing date for applications: February 12, 1979. 8844(A)

UNIVERSITY OF WARWICK RESEARCH FELLOW

Synchrotron Radiation Instrumentation

In the Department of Engineering to work with Dr D. K. Bowen on the design, construction and use of a computer-controlled X-ray topographic camera for use at the synchrotron radiation source under construction at S.R.C. Daresbury Laboratory.

Salary up to £4,382 per annum on the Range IA scale £3,883 to £6,555 per annum (under review). The post is tenable for three years, starting as soon as possible.

Application forms and further details from the Academic Registrar, University of Warwick, Coventry CV4 7AL. Closing date for receipt of applications January 15, 1979. Please quote reference 20/A/78. 8899(A)

NATURE CONSERVANCY COUNCIL

Senior Scientific Officer/ Principal Scientific Officer

SALARY SCALES

Senior Scientific Officer	**£5,154 to £6,898**
Principal Scientific Officer	**£6,609 to £8,461**
Plus Inner London Weighting	**£465 per annum.**

Applications are invited for the post of Senior Scientific Officer/Principal Scientific Officer in the N.C.C's Land Division.

The Land Division of the Nature Conservancy Council is based in London and headed by the Chief Land Agent. The Division is responsible to the Director, N.C.C. for Council's professional land agency and estate management throughout Great Britain.

THE POST

The successful candidate will be responsible for providing a specialist advisory service on land use economies and to monitor innovations and trends in commercial land use to assess the effects these may have on nature conservation. Principal duties will include examining and reviewing existing N.C.C. land management policy, maintaining an awareness of research into land use practices, and the dissemination of such knowledge to officers throughout the N.C.C.

AGE AND QUALIFICATIONS

Candidates should have a first or second class honours degree, or equivalent, in biology, botany, zoology, geology or geography and should also have subsequent training in agriculture or forestry with experience in, and knowledge of rural land use.

Senior Scientific Officer: Candidates must be at least 25 and under 32 years of age and have at least four years' relevant postgraduate experience.

Principal Scientific Officer: Candidates should normally be between 30 and 50 years.

Application forms and further details are available from Recruitment Section, Nature Conservancy Council, Godwin House, George Street, Huntingdon, Cambs., PE18 6BU (Tel. 0480-56191). Closing date for completed application forms is February 16, 1979. 8869(A)

SHEFFIELD AREA HEALTH AUTHORITY (TEACHING)
Centre for Human Genetics

CYTOGENETICISTS

There are immediate vacancies for two Cytogeneticists (Basic Grade) at the above unit. The work is part of a Regional diagnostic service and will include tissue culture and the identification of chromosome abnormalities. Applicants should have a relevant Science degree.

Salary Scale £3,486 rising to a maximum of £4,899. For further information telephone Sheffield (0142) 667333.

Applications giving details of age, qualifications and experience, with the names and addresses of two referees to: the District Personnel Officer, Floor B, Hallamshire Hospital, Glossop Road, Sheffield S10 2JF.

8880(A)

Graduate Biologist

Fungicide Screening

Chesterford Park Research Station is the Research and Development centre for the Agrochemical Division of Fisons Limited. We are looking for a graduate biologist to join the Fungicide Screening Team in the Biological Screening Department.

As a Scientist II you will supervise the work of two Research Assistants and participate in the laboratory evaluation of new compounds for their fungicidal (in-vitro only) and bacterial activity against plant pathogens.

This position will be suitable for a first degree graduate with an interest in, and preferably some practical experience of, working with plant bacterial diseases.

We offer a competitive starting salary and the substantial range of benefits associated with working for a major industrial company, including relocation expenses where appropriate.

For full details and an application form please write to or telephone: Roger Lake, Personnel & Training Officer, Fisons Limited—Agrochemical Division, Chesterford Park Research Station, Saffron Walden, Essex. CB10 1XL. Telephone: Saffron Walden 23542.

FISONS

8916(A)

GRANTS FOR BASIC RESEARCH:

New Approaches to Tropical Diseases

The Special Programme for Research and Training in Tropical Diseases is encouraging research into the problems of tropical diseases Its Biochemical Sciences Scientific Working Group has limited funds available for research proposals on basic mechanisms of parasitism in malaria, schistosomiasis, filariasis, trypanosomiases, leishmaniases and leprosy. The following areas have been defined as particularly relevant·

- —Parasite-host cell recognition and intracellular interactions
- —Identification of changes in the membrane of parasitised cells.
- —Isolation of parasite mutants and variants modified with respect to infectivity and/or pathogenicity.
- —Fractionation of parasites for the purpose of isolation of antigens and other molecules
- —Identification of host genetic factors which modify infection and influence the development of parasitic disease, including methods for HLA typing applicable under field conditions.
- —Elucidation of the activities of eosinophils and basophils in metazoan parasitic infection

However, proposals in other topic areas offering insights into the basic mechanisms of parasitism are also welcome.

The research proposals should be relevant to improved control of the six diseases of concern to the Special Programme Model systems elucidating these diseases or their pathogens will also be entertained

Enquiries and requests for research proposal forms should be addressed to.

Office of the Director
Special Programme for Research and
Training in Tropical Diseases
World Health Organisation
CH-1211 Geneva 27, Switzerland

Deadline for receiving the current cycle of applications is 15 April, 1979. 8935 (A)

UNIVERSITY COLLEGE OF SWAZILAND

Applications are invited for the post of LECTURER IN THE DEPARTMENT OF CHEMISTRY

Candidates should possess a Ph D in Chemistry with specialisation in Physical Chemistry Candidates with qualifications in inorganic chemistry will also be considered. The appointee will be concerned with first and second year teaching in the subject, with the possibility of third and fourth year teaching from 1980 onwards There will also be opportunities for research.

Salary scales· Lecturer I E5,940 to E7,860 per annum (£1 sterling=E1 68) Salary scale and entry point according to qualifications and experience. The British Government may supplement salary in range £1,650 to £2,184 per annum (sterling) for married appointee or £480 to £960 per annum (sterling) for single appointee (currently under review and normally free of all tax) and provide children's education allowances and holiday visit passages Short-term contracts for two or four years If appointment is for a limited period, a 25% gratuity is paid in lieu of superannuation for the first two years of service, 27½% for the second two years. A 10% inducement allowance is payable to expatriates not qualifying for supplementation from other sources. Family passages, education allowance for expatriates, free ordinary medical attention at Government institutions, accommodation at reasonable rental; biennial leave

Detailed applications (two copies) with curriculum vitae and naming three referees should be sent to the Registrar, University College of Swaziland, Southern Africa, by January 22, 1979. Applicants resident in the U K should also send one copy to the Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT Further details may be obtained from either address. 8923(A)

THE ROWETT RESEARCH INSTITUTE

Bucksburn, Aberdeen AB2 9SB

BIOMETRY DEPARTMENT

A vacancy exists for a Scientific Officer or Higher Scientific Officer. The principal duties are to participate in the development of the data-acquisition and processing system, based on an IBM system 7 and an IBM 1130 computer, to advise and assist members of the scientific staff in writing and running their programmes, and in the statistical analysis and interpretation of their experiments. Knowledge of Fortran IV and Assembler, together with some experience of an IBM 1130 or similar computer, an advantage

Candidates should have a first or upper second class honours degree in Computing Science.

The appointment will be as Scientific Officer (Salary £3,294 to £4,415 per annum) or Higher Scientific Officer (£4,101 to £5,448), grade and starting salary depends on experience At least two years' relevant post-graduate experience is required for Higher Scientific Officer grading.

There is a non-contributory pension scheme.

Further details of the post may be obtained from the Secretary of the Institute, to whom applications should be submitted together with the names and addresses of two referees. Closing date January 19, 1979 8875(A)

THE UNIVERSITY OF LIVERPOOL

HEATH HARRISON CHAIR OF ORGANIC CHEMISTRY

Applications are invited for the Heath Harrison Chair of Organic Chemistry.

The salary will not be less than £9,865 per annum and initially will depend on the qualifications and experience of the successful candidate.

Applications (12 copies), together with the names of three references, should be received not later than January 26, 1979, by the undersigned, from whom further particulars may be obtained (Candidates overseas may send one copy only by airmail). Quote Reference RV/427/N.

H H Burchnall, Registrar,
The University,
P O Box 147,
Liverpool L69 3BX 8901(A)

BIOPHYSICIST

A tenure track position will be available in Biophysics at the University of Illinois effective Fall 1979 The position is expected to be at the Assistant Professor level

It is anticipated that the successful applicant will carry out research and teaching in biophysics, in the general area of biomolecular structure Candidates should have a Ph D degree and some postdoctoral experience Salary is negotiable.

Please send your curriculum vitae by March 1, 1979, to Dr A. R Crofts, Chairman of Biophysics, 524 Burrill Hall, University of Illinois, Urbana, IL 61801 (Tel 217-333-7407)

The position is conditional upon continued availability of funds. **An Equal Opportunity and An Affirmative Action Employer** 8902(A)

PAISLEY COLLEGE

RESEARCH ASSISTANT

PHYSICS DEPARTMENT

This is an S R C funded R.A 1B post involving the dynamic measurement of oil film thickness in tribology by neutron scanning using an image intensifier. The project will involve work at the East Kilbride S U R R C nuclear reactor facility and is in collaboration with the National Engineering Laboratory.

Applications are invited from post-doctoral or experienced postgraduate engineers/physicists The initial contract will be for two years, at a salary in the range £3,500 to £4,000 per annum

Applicants should write to Dr. A. R Spowart, Senior Lecturer, Physics Department, Paisley College of Technology, High Street, Paisley PA1 2BE, attaching a curriculum vitae with the names of two referees, by February 28, 1979. 8903(A)

WESSEX WATER AUTHORITY
REGIONAL BIOLOGY UNIT

MARINE BIOLOGIST

£4,635—£4,962 per annum including supplement plus car allowance

Applications are invited for the above post within the Regional Biology Unit at the Regional Biology Laboratory, Corfe Mullen, near Wimborne, Dorset

Duties include organising and carrying out routine Marine Biological Monitoring and special Marine Biological investigations of Estuaries and Coasts, the two coastlines of the Wessex Water Authority area, and also involve working at sea on the Authority's research vessel.

Applicants must be suitably qualified Marine Biologists and ideally should have experience of Marine Monitoring it would also be an advantage to be an experienced Sub-Aqua Diver Applicants must also hold a current driver's licence.

A regular car user allowance will be paid, together with re-location expenses where applicable.

Application forms and job description, quoting reference number 362/54 are obtainable from Mr T Pleass, Personnel Officer, Wessex Water Authority, Wessex House, Passage Street, Bristol (telephone Bristol 290611 Ext 229) and should be returned no later than 16th January 1979 8915(A)

Wessex Water

NEW SOUTH WALES STATE CANCER COUNCIL

SPECIAL UNIT FOR INVESTIGATION AND TREATMENT

BIOMETRICIAN

The Special Unit of the New South Wales State Cancer Council, situated at the Prince of Wales Hospital, Sydney, Australia, requires the services of a biometrician to take charge of statistical aspects of the activities of both the Council and the Special Unit Of special interest is the organisation and interpretation of clinical trials, laboratory results, population surveys and the medical records of cancer patients

At present, statistical assistance is provided by a senior consultant, and it is expected that this relationship will be maintained and fostered Facilities currently available within the Unit are a time sharing facility via a DIABLO 1620 console linked to a CYBER 72 CDC at the University of N S W., and a small WANG 2000 computer.

The position requires someone with a good personality and a high degree of initiative. Postgraduate qualifications are essential and practical experience in statistics and computing is desirable

Salary will be that of a Senior Scientific Officer ($16,686 to $20,409) Conditions of employment in accordance with Scientific Officers (Public Hospital Scientists) Award

Applications should contain full details of personal background, qualifications and experience, and, where appropriate, a curriculum vitae

Further information may be obtained from Dr A R Nash, Special Unit, Prince of Wales Hospital, Randwick, Australia 2031 Written applications to be forwarded to above address 8917(A)

DEVELOPMENTAL BIOLOGIST

Applications are invited for a faculty position to begin September, 1979 Candidates should have Ph D in appropriate field and postdoctoral experience Rank and salary are open

Duties include teaching at undergraduate and graduate levels and development of a vigorous research programme.

Send curriculum vitae, statement of research plans, copies of publications, and arrange for three letters of recommendation to be sent by January 15, 1979 to.

Professor G M. Happ,
Department of Zoology,
University of Vermont,
Burlington, Vermont 05405

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THAI NATIONALS

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8717(A)

POSTDOCTORAL POSITION

of two-year duration available immediately for sequence and affinity labelling studies on ribulosebisphosphate carboxylase at salary of $12,000 for first year

Please send resume and letters of references to. Dr Fred C Hartman, Oak Ridge-University of Tennessee Graduate School of Biomedical Sciences, P O Box Y, Oak Ridge, TN 37830 *An Equal Opportunity/Affirmative Action Employer.* 8908(A)

CSIRO

AUSTRALIA

Postdoctoral Research Fellow

Division of Irrigation Research Griffith, NSW

CSIRO has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 7,000 employees —2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia

Field: Plant Nitrogen Studies

General The Division is located at Griffith, about 600 km north of Melbourne and 650 km west of Sydney. Griffith has a population of about 13,000 and is situated in a well-developed rural centre, with good educational and extensive recreational facilities. The Division's research encompasses a wide range of disciplines within the context of irrigated cropping, including studies on soil-root zone biology and environmental plant physiology and biochemistry

Duties: To join a research group concerned with metabolic factors which limit the efficiency of nitrate utilisation by crop plants The work will relate to how plants integrate biochemical fluxes of carbon and nitrogen metabolites so that some experience with isotopic technique (^{14}C and ^{15}N) would be an advantage.

Qualifications A Ph.D degree or equivalent qualifications in plant physiology or biochemistry Experience with ^{15}N techniques would be an advantage.

Salary: Research Scientist or Senior Research Scientist A$14,829—$A21,543 p.a.

Tenure: A fixed term appointment of three years.

Applications IN DUPLICATE, stating FULL personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 500/345 should reach.— The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, LONDON WC2R 3EH by 31st January 1979

Applications in U S A. and Canada should be sent to:—The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W , WASHINGTON, D C , 20036, U.S A.

8929(A)

AN FORAS TALUNTAIS

Requires a

GRADUATE IN BIOCHEMISTRY, FOOD SCIENCE or VETERINARY SCIENCE

For up to 3 years to undertake a project funded by the Pig Industry, involving research on the measurement of quality in Irish Pigmeat.

ESSENTIAL

Either, an Honours University degree, or equivalent, in Biochemistry, Food Science, or a related discipline OR a degree in Veterinary Science or equivalent.

DESIRABLE:

A higher degree and/or suitable research experience preferably in the Food Industry

Application form and further particulars may be obtained from the Personnel Department, An Foras Taluntais Headquarters, 19 Sandymount Avenue, Ballsbridge, Dublin 4 ('Phone Dublin 688188). Latest date for receipt of completed forms, Monday, January 22, 1979. 8934(A)

OVERSEAS DEVELOPMENT

KNOW-HOW: vital to developing countries

Agronomist/ Crop Protection SUDAN

To work as part of a Food Crop Development Team within the Project Development Unit of the Regional Ministry of Agriculture, Juba. Specific responsibility for 1. Identification of major pests diseases and weeds of economic importance in the Southern Region. 2. The conducting of field trials aimed at control of pests, diseases and weeds in crops. 3. Screening of local and imported plant material for pest and disease resistance. Applicants aged 25-35 years should have a degree in agriculture and at least 3 years experience, preferably overseas in agronomy and crop protection.

Appointment 2 years. Salary (UK taxable) in range £4,100-£7,500 p.a. plus tax free overseas allowance in range £2,145-£4,725 p.a. (Ref. 331X).

The post is wholly financed by the British Government under Britain's programme of aid to the developing countries. In addition to basic salary and overseas allowances other benefits normally include paid leave, free family passages, children's education allowances and holiday visits, free accommodation and medical attention. Applicants should be citizens of the United Kingdom.

For full details and application form please apply, quoting reference stating post concerned, and giving details of age, qualifications and experience to:

Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London SW1E 5DH. 8912(A)

ODM

HELPING NATIONS HELP THEMSELVES

UNIVERSITY OF QUEENSLAND
Australia
PROFESSOR OF PHARMACY

Applicants must have pharmaceutical qualifications acceptable for registration within the State of Queensland and appropriate higher degrees. They should present evidence of significant research achievement, have substantial teaching experience and appropriate professional experience. While the University would welcome applications from persons experienced in any area of pharmacy preference may be given to applicants with a particular interest in Biopharmaceutics.

The present salary for a Professor is $A31,789 per annum. The University provides prescribed travelling and removal expenses, superannuation, housing assistance and study leave (currently under review).

Additional information and application forms are available from the Secretary General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF. Applications close on March 31, 1979. 8888(A)

PRINCETON UNIVERSITY
DEPARTMENT OF BIOLOGY

Seeks applicants for the position of.

ASSISTANT PROFESSOR

in the field of population biology. Preferential consideration will be given to candidates whose interests overlap and complement those of existing members of Princeton's population biology group: Gould, Horn, May and Terborgh Appropriate areas are. the ecology and adaptive behaviour of animals and plants, population dynamics, sociobiology and biogeography Field experience and a wide ranging interest in organisms are desirable qualifications. Evidence of exceptional promise in research is essential. Teaching duties will consist of one undergraduate and one graduate level course and counselling students in research Salary competitive, to be negotiated Applicants should send a curriculum vitae, a statement of research experience and interests, and three letters of recommendation to: Dr John Terborgh, Department of Biology, Princeton University, Princeton, New Jersey, 08540.

Equal Employment Opportunity/ Affirmative Action Employer, M/F 8783(A)

THE UNIVERSITY OF MANCHESTER
FACULTY OF MEDICINE
VETERINARY OFFICER

A veterinary graduate with experience in the care and use of laboratory animals is required to manage experimental animal facilities for the Faculty These will comprise the animal floor of the New Medical School building, extending to about 36,300 sq. feet and an outstation for larger animals and for certain breeding operations In addition there are separate University-supervised animal facilities at the University Hospital of South Manchester and Hope Hospital, Salford, for which the veterinary officer will be responsible These facilities cater for all the requirements in the Faculty, including clinical departments The appointee will be encouraged to pursue his or her own research

Salary range per annum: £7,508 to £9,263. Superannuation.

Particulars and application forms (returnable by January 15) from the Registrar, The University, Manchester M13 9PL Quote reference 276/78/N 8881(A)

THE QUEEN'S UNIVERSITY OF BELFAST
RESEARCH ASSISTANT
DEPARTMENTS OF ARCHAEOLOGY AND BIOCHEMISTRY

A Research Assistant is require
to investigate the state of molecula
preservation of bone collagen fro
archaeological contexts to test th
feasibility of gaining palaeogeneti
information concerning earl
domestication and selective bree
ing The work will be carried ou
in the Department of Biochemistr
and will be jointly supervised b
Professors E M Jope (Archae
ology) and D. T. Elmore (Bio
chemistry) Experience in protei
sequencing and electron microscop
is desirable.

The appointment is for two year
from February 1, 1979 or as soo
as possible thereafter and wi
probably include a consultativ
visit to the U.S.A. Technica
assistance will be provided. Salar
range (under review) £3,384 t
£3,885 with U.S.S., initial placin
depending on age, qualification
and experience.

Applications, together with th
names and addresses of thre
referees, should be sent to th
Personnel Officer, The Queen'
University of Belfast BT7 1NN
Northern Ireland. Closing date
January 20, 1979 8874(A)

ST BARTHOLOMEW'S HOSPITAL
London EC1A 7BE
RESEARCH TECHNICIAN

required for radioimmunoassay o
hormones in blood The successfu
candidate will provide a service fo
the research projects within th
Department of Endocrinology. Pre
vious experience in radioimmuno
assay useful but not essential
Ability to work without supervisio
essential. Applicants should b
state registered and possess H.N.C.
or equivalent. Whitley Counci
salary and conditions of service.
This post is tenable until March
1980 in the first instance.

Please telephone 01-600 9000, ext. 3320 for an application form quoting ref. RP 90/88.

8882(A)

UNIVERSITY OF BRISTOL

Applications are invited
for the post of

LECTURER IN ANATOMY

A vacancy exists for a lecturer wi
a primary responsibility to the teac
ing of medical students. As well
being responsible for the teaching
gross anatomy to medical, dent
veterinary and science students, t
Department offers courses in neur
anatomy, endocrinology, reprodu
tion, embryology, dental anatomy a
physical anthropology, with speci
ised teaching in each of these top
to an Honours degree level.

The Department has excelle
facilities for research and is present
organised into a number of resear
groups working in the following are
biochemistry and physiology of pe
tide-secreting neurones, neroanato
of dopamine pathways, electrophys
logy of the CNS, control of partu
tion, bone mechanics, tooth eruptio
and physical anthropology.

Salary will be on the scale £3,8
to £7,754 (under review) and letters
application including the names a
addresses of three referees should
sent to the Registrar and Secreta
University of Bristol, Senate Hou
Bristol BS8 1TD, before February 2
1979 (quoting ref. TLJ). Interest
persons are welcome to visit the D
partment by arrangement with t
Head of Department's secretary (T
0272-24161, Ext. 367). 8895(

PLANT BREEDING INSTITUTE

Maris Lane, Trumpington
Cambridge CB2 2LQ

HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER

CYTOGENETICS DEPARTMENT

A Cell Biologist is required for a period of three years to carry out a feasibility study of transferring DNA into cereals and related species using techniques involving cell or tissues other than cell protoplasts where whole plant regeneration may be a problem. Candidates should have a 1st or Upper 2nd Class Honours Degree in an appropriate biological science and at least two years' postgraduate experience in cell biology or related research including practical experience of micromanipulation techniques.

The appointment will be as Higher Scientific Officer (£4,101 to £5,448 per annum) or Senior Scientific Officer (£5,154 to £6,898 per annum). At least four years' relevant postgraduate experience is required for appointment as Senior Scientific Officer.

Applications with curriculum vitae and the names and addresses of three referees should be sent to the Assistant Secretary (Establishment) by January 8, 1979.

Please quote reference CYT/34.

8937(A)

PLANT BREEDING INSTITUTE

Maris Lane, Trumpington
Cambridge CB2 2LQ

HIGHER SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER

CYTOGENETICS DEPARTMENT

A Molecular Biologist is required to participate in and be responsible for parts of a programme of studying plant chromosomes, isolating plant genes and producing vectors and specific nucleotide sequences for the transformation of plant cells.

Candidates should have a 1st or Upper 2nd Class Honours Degree in an appropriate biological science and at least two years' postgraduate experience in molecular biology or related fields of biochemistry.

The appointment will be as Higher Scientific Officer (£4,101 to £5,448 per annum) or Senior Scientific Officer (£5,154 to £6,898 per annum). At least four years' postgraduate experience is required for appointment as Senior Scientific Officer.

Applications with curriculum vitae and the names and addresses of three referees should be sent to the Assistant Secretary (Establishment) by January 8, 1979.

Please quote reference CYT/35.

8936(A)

SURFACE PHYSICIST/CHEMIST

Fusion energy research on plasma-wall interaction phenomenon involving atomic hydrogen chemical sputtering and surface cleaning. Uhv and surface physics/chemistry experience appropriate. Appoint at Research Associate or Senior Research Associate level. Appointment for three years initially.

Well-qualified scientist sought. Appropriate salary available.

Write: Professor P. C. Strangeby, University of Toronto, Institute for Aerospace Studies, 4925 Dufferin Street, Downsview, Ontario M3H 5T6. (416) 667-7729. 8907(A)

UNIVERSITY OF SHEFFIELD

LECTURER IN HUMAN METABOLISM AND CLINICAL BIOCHEMISTRY

Applications are invited for men and women for the post of Lecturer in the Department of Human Metabolism Clinical Biochemistry, based in the Hallamshire Hospital and Medical School. The department has responsibilities for teaching medical students and has excellent facilities for research both in basic science and on clinical projects. Current research interests include calcium and phosphate metabolism, Vitamin D, the biochemistry of platelets, peptide hormone receptors and abenylate, cyclase, cyclic nucleotide metabolism, endocrine aspects of cancer, and pathogenic mechanisms in arthritis. A medical degree would be preferred but is not essential. Initial salary in the range of £3,883 to £6,080 (non-clinical) or £4,298 to £6,935 (clinical). Further information available from Professor R. G. G. Russell (0742 26484, ext. 3037), Department of Human Metabolism Clinical Biochemistry, University of Sheffield Medical School, Beech Hill Road, Sheffield F10 2RX, and from The Registrar and Secretary, The University, Sheffield F10 2TN.

Applications to the Registrar and Secretary by January 12, 1979. Quote ref: 236/G. 8933(A)

UNIVERSITIES OF MANCHESTER AND LIVERPOOL

·Universities Research Reactor, Risley, near Warrington

EXPERIMENTAL OFFICER (ASSISTANT ANALYST)

in the Activation Analysis Service

Applications are invited for the post of EXPERIMENTAL OFFICER (ASSISTANT ANALYST) in the Activation Analysis Service of the Universities Research Reactor. The successful candidate will be expected to plan and carry out trace element analysis of a wide range of materials submitted to the Service by Industrial and University research workers. The techniques usually involved are those of thermal neutron activation; other radiochemical techniques are used when necessary. Facilities available include the 30 kW nuclear reactor on the site, a fully equipped Radiochemical Laboratory, comprehensive detection and nucleonic equipment. Candidates should have a sound knowledge of inorganic chemistry; experience in inorganic analysis or radiochemistry would be an advantage, together with at least pass degree or equivalent in chemistry.

Salary range: £3,384 to £5,604 per annum. U.S.S.

Particulars and application forms (returnable by January 20) from the Registrar, The University, Manchester M13 9PL. Quote ref: 277/78/N.

8924(A)

UNIVERSITY OF OXFORD

LECTURESHIP IN ASTRONOMY

Applications are invited, from men and women candidates, for a University Lectureship in Astronomy, in the Department of Astrophysics, to work in the field of observational astronomy, having particular regard to the theoretical interpretation of observational data, to be filled from October 1, 1979.

The stipend will be according to age on the scale (under review) £3,883 to £8,257 with superannuation. The lectureship may be held with a tutorial fellowship in physics at St. Hugh's College and a linked non-stipendiary lectureship at St. Edmund Hall.

Further details may be obtained from Professor D. E. Blackwell, Department of Astrophysics, South Parks Road, Oxford OX1 3RQ, to whom applications (one typed copy), including a statement of qualifications and publications and the names of three referees, should be sent before February 7, 1979. 8883(A)

STRATHCLYDE
REGIONAL COUNCIL

DEPARTMENT OF EDUCATION
FURTHER EDUCATION

Applications are invited for the undernoted post at Glasgow College of Technology, Cowcaddens Road, Glasgow G4 0BA.

SENIOR LECTURER 'A' IN OPHTHALMIC OPTICS

The Senior Lecturer will be required to contribute to the teaching and supervision of the BSc with Honours in Ophthalmic Optics and other courses in the Ophthalmic area. Research and consultancy are encouraged. Interests in ocular anatomy, visual science or clinical ophthalmic studies will be an advantage. Applicants should possess relevant experience and either a degree and professional qualifications in Ophthalmic Optics or a degree in a relevant discipline such as physics or physiology together with a research degree and an appropriate record of research in visual or ophthalmic science. Teacher training would be an advantage but training can be given on an in-service basis.

Salary Scale:

SENIOR LECTURER 'A' .. £7,098-£7,899 Bar—£8 967

Placing on the salary scale will be given for relevant experience.

Forms of application and further particulars can be obtained from the College to whom completed application forms should be returned not later than January 20th, 1978.

(Edward Miller, Director of Education)

8877(A)

STATE OF KUWAIT
KUWAIT UNIVERSITY

Advertisement for Environmental/Geomorphology—Geology Department

The Department invites applicants for a two-years renewable appointment starting September 1979. Qualifications include a Ph.D., experience in teaching environmental geology and geomorphology and willingness to participate (on rotating basis) in instructing elementary courses in marine, geo-statistics and introductory Geology. The candidate will be expected to assist in developing research programmes in Geology and teaching in the Graduate School which opens shortly. Applicants should send résumé and three letters of reference to Kuwait University, Department of Administrative Affairs, P.O. Box 5969, Kuwait. 8885(A)

Geologists &
Geophysicists

Oil and Gas Exploration in Ira

OSCO — The Oil Service Company of Iran, a consortium of 14 international oil companies, is engaged in a major hydrocarbon exploration and development programme on behalf of the National Iranian Oil Company.

In order to accomplish this task we seek to strengthen our team of senior professionals by making the appointments detailed below.

Initial contracts 2 years. Salaries according to seniority and experience are paid in US dollars nett of Iranian income tax. Benefits include married or single status accommodation, free medical facilities, life insurance, educational assistance and a bonus on termination of contract.

Stratigraphic Group Head
c. $40,000

Responsible to the Chief Stratigrapher, you will be leading a group of specialist geologists in studies of a specific stratigraphic interval of rock in operating areas to ensure optimal exploration programmes. You will liaise closely with other specialist groups and with exploitation geologists. *(Ref T558/N)*

Area Geologist – Sub Surface
c. $40,000

To supervise and direct the exploration activities in an assigned area with the object of identifying the hydrocarbon reserves to meet offtake and capacity requirements. You will directly supervise a Project Leader and a Senior Geologist and, indirectly, the work of up to ten specialists. *(Ref T559/N)*

Project Leader – Stratigraphy
c. $36,000

Responsible for leading geological survey parties to make regional studies of the area of operation to obtain new data and to review the total geological information available and to make detailed recommendations for exploration drilling. *(Ref T568/N)*

Project Leader – Sub Surface
c. $36,000

A senior member of the team working under the direction of the Area Geologist, with the goal of finding the reserves to meet offtake and capacity requirements. You will obtain and analyse data and make recommendations for wildcat and exploration wells. *(Ref T565/N)*

$34,000 to $40,000 p.a. nett

Project Leader – Geophysics Operations
c. $36,000

Reporting to the Chief Geophysicist, you will be responsible for the planning and supervision of seismic parties, comprising field surveys, digital processing and interpretation. You will need to be able to advise on the most up-to-date field techniques. *(Ref T553/N)*

Project Leader – Interpretation
c. $36,000

Responsible to the Area Geophysicist for ensuring that proper appraisals are made of all geophysical data over all prospects and that the final compilation of maps prepared includes all pertinent geological and geophysical data. *(Ref T544/N)*

Senior Geologist –
c. $34,000

Reporting to an Area Geologist, you will be obtaining and analysing the geological data necessary for selecting and evaluating the locations for exploration wells. *(Ref T567/N)*

Data Acquisition Specialist
c. $34,000

Reporting to the Chief Geophysicist, you will act as adviser and consultant to the geophysical department on acquisition techniques and systems, so as to obtain data in the optimum manner, which after processing and interpretation will lead to a better geological understanding of the sub surface. *(Ref T566/N)*

Senior Geophysicist
c. $34,000

You will be assisting a Project Leader to plan, supervise and interpret the work of seismic contractors and preparing compilation maps for all seismic horizons of interest. *(Ref T537/N)*

Senior Geologist – Regional Studies
c. $34,000

You will be engaged in the conduct of regional studies in the area of operation and its adjacent areas by obtaining new data and reviewing existing information in the fields of stratigraphy, paleontology and related disciplines, in the search for deeper structural prospects. *(Ref T547/N)*

Candidates for these appointments must be university graduates in relevant subjects, who have had at least eight years' experience related to oil and gas exploration. Advanced degrees in specialist subjects are particularly advantageous. The preferred age range is 32-45 years.

Please write giving full details of your age, education, career history and salary progresison to date, quoting the appropriate reference number, to:

F. R. Wilcockson, Director,
Whites Recruitment Limited,
72 Fleet Street, London EC4Y 1JS.

IRANIAN OIL SERVICES LTD.

CSIRO

AUSTRALIA

Research Scientist

Division of Plant Industry Canberra, ACT

CSIRO has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: Molecular Basis of Plant Improvement

General: The Division conducts pure and applied research in molecular biology, genetics, plant breedings, genetic resources, physiology, microbiology, biochemistry, plant nutrition, ecology, crop adaptation, soil fertility and plant introduction.

This position is in a research programme concerned with the molecular basis of plant improvement. The ultimate objective being to extend the scope of plant breeding by permitting specific genetic manipulation of the plant genome.

Duties: To participate in a research programme in plant molecular biology aimed at achieving the transfer and expression *in vivo* of isolated plant genes and in examining the structure and function of plant genomes.

Qualifications: Applicants should have a Ph.D. degree in an appropriate field or postgraduate research experience of equivalent standard or duration supported by satisfactory evidence of research ability. Previous research experience in recombinant DNA methodology and in its application to the analysis of plant genomes is desirable. Preference will be given to applicants who also have expertise in plasmid genetics.

Salary: Research Scientist or Senior Research Scientist: $A14,829—$A21,543 p.a.

Tenure: Indefinite with superannuation.

Applications in DUPLICATE, stating FULL personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 130/1437 should reach:—The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, LONDON WC2R 3EH by 19th January 1979.

Applications in U.S.A. and Canada should be sent to:—The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., WASHINGTON, D.C., 20036, U.S.A.

8928(A)

DAMMAM SAUDI ARABIA

KING FAISAL UNIVERSITY

COLLEGES OF MEDICINE AND MEDICAL SCIENCES

TEACHING AND HOSPITAL APPOINTMENTS

FOR MEN AND WOMEN

The expanding Colleges of Medicine and Medical Sciences invite applications from suitable qualified men and women for teaching and teaching/clinical appointments. The curriculum follows American pattern of education, and the languages of instruction is English.

In 1979—1980 courses will cover the following subjects:

General Chemistry
Organic Chemistry
Physiology
Anatomy
Embryology
Physics
Biology
Cell Biology
Biochemistry
Pathology
Haematology
Pharmacology

Community Medicine
Epidemiology/Biostatistics
Orthopaedics
Immunology
Parasitology
Microbiology
General Surgery
Internal Medicine
Obstretrics/Gynecology
Paediatrics
Orhthalmology
Otorhinolaryngology

	Monthly salary scales in Saudi Riyals	*Approximate annual Sterling equivalents*
Professor ..	SR 7,195–9,595	£13,500–£18,000
Associate Professor ..	SR 6,435–8,185	£12,000–£15,300
Assistant Professor ..	SR 5,425–7,765	£ 9,800–£14,500

Medical Degrees attract a 30% bonus.
Hospital Duties attract a 80% bonus.
Annual home leave travel allowances are offered.
There is no income tax payable to the Saudi Arabian Government.
Free furnished accommodation is provided.
Assistance with childrens' education costs.
Interviews will be held in the United Kingdom and in the United States in late February 1979. Send a curriculum vitae to: Dr. Tawfik Tamimi, M.D., Dean

P.O. Box 2,
Sunbury-on-Thames,
Middlesex,
England.

or King Faisal University,
Saudi Arabian Educational Mission,
2223 West Loop South,
Houston, Texas 77027, U.S.A.

8930(A)

NIVERSITY OF LEICESTER

-RAY ASTRONOMY GROUP

OMPUTER OFFICER

k on the UK-6 and HEAO-B tell projects in the X-Ray Astronny Group. The successful candidate l be required to take responsibility the operation of the Data Terminal l to assist with writing and maintene of data analysis software for n UK-6 *and* HEAO-B projects. didates *should* be graduates in sics or Astrophysics, with experiof computer programming/ations (particularly with PDP-11 ns).

E appointment is until December (subject to satisfactory comple- a one-year probationary period), alary on the Research Assistant 1B £3,384 to £4,882.

LICATIONS in writing, with rriculum vitae and names of ferees, to Professor K. A. Department of Physics, University, University Road, LE1 7RH, by January 5.

8879(A)

UNIVERSITY OF QUEENSLAND

PHYSICAL CHEMIST/PHYSICIST/MATERIAL SCIENTIST

SENIOR RESEARCH ASSISTANT GRADE III—CHEMISTRY

Experimental work under direction on electro-deposition of semiconductor films and for testing of solar cells. Honours degree or good pass degree required. Appointment for one year with possibility of renewal.

Salary according to experience in the range $A11,667 to $A12,505 per annum. Some travel expenses could be paid.

Applications should be forwarded to the Staff Officer, University of Queensland, St. Lucia, Qld. 4067, Australia, and applicants should ask two referees to write immediately on their behalf to the Staff Officer. Quote Reference No. 43478.

8920(A)

UNIVERSITY OF BRISTOL

Applications are invited for the post of

RESEARCH ASSISTANT/ASSOCIATE

Within the Department of Anatomy. The post is tenable for up to three years and the appointee will join Profesor B. T. Pickering's group studying the biosynthesis, ultraneuronal transport and release of neurohypophysical polypeptides. Applicants should have some experience of biochemical, pharmacological, ultrastructural or immunocytochemical techniques; and be in possession of either a Ph.D. or a medical qualification.

Salary will be on the scale £3,883 to £6,555 (under review). Letters of application including curriculum vitae and the names of **three** referees should be sent to the Registrar and Secretary, University of Bristol, Senate House, Bristol BS8 1TH before January 31, 1979 (quoting ref. TLJ).

8896(A)

UNIVERSITY OF NEWCASTLE UPON TYNE

POSTDOCTORAL RESEARCH ASSOCIATE

Applications are invited for the post of S.R.C. Postdoctoral Research Associate to work on the kinetics of the coupled transport of β-galactosides and protons into *Escherichia coli.* Applicants with experience of pre-steady-state kinetics are particularly wanted but experience in the fields of membrane transport or bioenergetics would also be considered relevant. Further details may be obtained from Dr I. C. West.

The appointment will be for three years, starting as soon as possible. The starting salary will be at the appropriate point on the 1A Research Associate scale (£4,382 at age 26, under review).

Applications, including a curriculum vitae and the names of two referees, should be sent to Dr I. C. West, Department of Biochemistry, The University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU. 8913(A)

DIRECTOR
INSTITUTE OF MARINE RESOURCES
PROFESSOR
SCRIPPS INSTITUTION OF OCEANOGRAPHY

Senior scientist with experience in innovation investigations related to marine resources and management thereof, to lead research groups at Scripps and University of California, Davis; prepare budgets for University funds and assist in obtaining extramural funds; administer statewide Sea Grant College Program; and teach graduate students. Appointed by the President, University of California. Salary (state funded) to $44,000 for 11 months, depending on qualifications. Send vitae and names of references by 30 January 1979 to M. M. Mullin, A-008, IMR Search, University of California, San Diego, La Jolla, CA 92093. The University of California is an Equal Opportunity/Affirmative Action employer.

Please bill the University of California, San Diego, Accounting Office, Q-027, La Jolla, CA 92093, in triplicate. Reference budget No. 1655. 8893(A)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF BIOCHEMISTRY

Applications are invited from

ORGANIC CHEMISTS/ BIOCHEMISTS

for TWO POSTS of

POSTDOCTORAL SENIOR RESEARCH ASSISTANT

tenable for a period up to three years:

(A) for work on the biosynthesis of carotenoid pigments in micro-organisms. The work will involve the use of stable isotopic labelling (^{13}C and ^{2}H) and/or the development of enzyme systems.

(B) to investigate the biosynthesis and metabolism of the ecdysteroids in insects. Experience of steroid or terpenoid chemistry will be an advantage.

The appointments are tenable from a date to be arranged at an initial salary within the range £3,883 to £4,382 per annum plus U.S.S. Benefits.

Applications, together with the names of two referees, should be received as soon as possible by:

The Registrar,
The University,
P.O. Box 147,
Liverpool,
L69 3BX

from whom further particulars may be obtained. Quote Ref: RV/430/N. 8900(A)

UNIVERSITY OF MANCHESTER
DEPARTMENT OF MEDICINE

A Research Assistant is required to work on the assay of vitamin D metabolites in a research group. This is a temporary appointment for 15 months from January 1979 during the leave of absence of a member of the group. Applicants should have experience of competitive binding assay or radio-immunoassay, and should preferably hold a Ph.D. degree, but other graduates with relevant experience may be considered.

Salary ranges per annum: £3,883 to £4,382 (postdoctoral) or £3,384 to £4,382 (postgraduate).

Applications as soon as possible to Professor S. W. Stanbury, Department of Medicine, Manchester Royal Infirmary, Manchester M13 9WL. 8905(A)

SEISMIC DATA PROCESSING SPECIALIST

c $39,000 p.a. nett
Oil and Gas Exploration in Iran

OSCO – The Oil Service Company of Iran, a consortium of 14 international oil companies – is engaged in a major hydrocarbon exploration and development programme in South West Iran on behalf of the National Iranian Oil Company.

This specialist will be responsible to the Chief Geophysicist for planning and directing all seismic data processing activities, including the work of contractors' processing centres. You will act as adviser and consultant to operations geophysicists and geologists and promote an awareness of new and developing processing techniques in the industry.

You will hold a degree in Physics or Mathematics and have had at least eight years' professional experience of the practice of Geophysics, with the emphasis on seismic data processing. You will need to be able to demonstrate well developed decision-making and communicative skills.

Initial contract is for 2 years. Benefits include married or single status accommodation, free medical facilities, life insurance, educational assistance and a bonus on termination of contract. Salaries are paid in US dollars nett of Iranian income tax.

Please write giving full details of your age, education, career history and salary progression to date, quoting reference *T569/N* to:
F. R. Wilcockson, Director,
Whites Recruitment Limited,
72 Fleet Street, London EC4Y 1JS.

IRANIAN OIL SERVICES LTD. 8927(A)

BERKELEY PHYSICS

The PHYSICS DEPARTMENT of the University of California, Berkeley, expects during the next two years to be making one or two appointments at the Assistant Professor level. Preferred areas of research are astrophysics, atomic and plasma physics, condensed matter physics, nuclear physics, and particle physics, with some priority given to theoretical condensed matter physics, experimental plasma physics, and theoretical particle physics.

Applicants should write before February 1, 1979, to the **Chairman of the Physics Department, University of California, Berkeley CA 94720, U.S.A.**, giving curriculum vitae, publication list, and references. The University of California is an Affirmative Action Employer. 8867(A)

UNIVERSITY OF EXETER

A vacancy has arisen in the
DEPARTMENT OF BIOLOGICAL SCIENCES

for a Technical Grade 3 to work in Plant Physiology.

Applications are invited from persons with ONC Sciences or equivalent qualifications and normally three years' experience.

Salary £2,688 to £3,060 per annum (under review).

Letters of application giving details of age, qualifications and experience, and naming two referees should be sent to Miss Doreen B. Watson, University of Exeter EX4 4QJ, by January 12, 1979. Please quote reference No. 5106. 8870(A)

EVOLUTIONARY BIOLOGIST

Applications are invited for a faculty position to begin September, 1979. Ph.D. required in appropriate discipline (genetics, ecology, behaviour, morphology, taxonomy, physiology) and postdoctoral experience desirable. Rank and salary open.

Duties include teaching at undergraduate and graduate levels and development of vigorous research programme.

Send curriculum vitae, statement of research plans, copies of publications, and arrange for three letters of recommendation to be sent by January 15, 1979 to:

Professor G. M. Happ,
Department of Zoology,
University of Vermont,
Burlington, Vermont 05405.

An Affirmative Action Employer.
8925(A)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF BIO-MEDICAL PHYSICS AND BIO-ENGINEERING

RESEARCH ASSISTANT

Applications are invited for this post in a small group working on an evaluation of properties of short-term cultures of human tumour biopsy specimens with particular reference to their chemosensitivity. Applicants should have a degree in one of the biological sciences Experience in either tissue culture or cancer research would be advantageous.

The post, funded by Cancer Research Campaign, is tenable for nine months in the first instance from January 1, 1979 Extension for a further year is likely, subject to satisfactory progress.

Salary £3,883 to £4,382 on Range 1A Research and Analogous Staff Scale, with appropriate placing

Further particulars from The Secretary, The University, Aberdeen, with whom applications (two copies) should be lodged by January 12, 1979

8887(A)

MICROBIOLOGY UNIVERSITY OF TORONTO

Scarborough College

Division of Life Sciences

Applications are invited for an

ASSISTANT PROFESSORSHIP

in the tenure-stream The applicant should have a Ph D and post-doctoral research experience in microbiology. No preference exists for any particular research specialty within the field Demonstrated ability to teach effectively across the subject area would be an important asset. Principal duties would be undergraduate teaching, participation in graduate instruction and the development of an active research programme.

The current salary floor is $16,500

The apopintment is open from July 1, 1979, and the applicant must be available to commence duties not later than September 4, 1979. Curriculum vitae and the names of three referees should be sent to Dr G. R Williams, Chairman, Division of Life Sciences, Scarborough College, University of Toronto, West Hill, Ontario MIC 1A4.

8886(A)

THE MOUNT DESERT ISLAND BIOLOGICAL LABORATORY

(M D.I B.L)

welcomes applications from established Investigators wishing to join its year-round programme.

Work at M D.I.B L has centred around problems of comparative physiology. A wide range of relevant research interests will be considered, including cell biology, environmental toxicology, developmental biology, and physiology

Independent grant support desirable. Curriculum vitae and correspondence should be addressed to:

Dr. Bodil Schmidt-Nielson,
Chairperson, Search Committee,
Mount Desert Island Biological Laboratory,
Salsbury Cove, ME 04672 U.S A

8890(A)

RESEARCH TECHNICIAN for Department of Zoology and Comparative Physiology for maintenance of research cultures of insects and assistance in research on insects nervous systems, sense organs and behaviour. Salary scale £2,688 to £3,060 per annum Reference 141/C/433 Application form from: Assistant Secretary, Personnel Office, University of Birmingham, P O. Box 363, Birmingham B15 2TT.

8884(A)

COURSES

EMBO COURSE

European Molecular Biology Organisation

Carlsberg Laboratory Copenhagen

May 7—13, 1979

Chromosome pairing and crossing-over

The laboratory exercises will consist in serial sectioning of meiotic nuclei, three-dimensional reconstructions of synaptinemal complexes from electron micrographs and analyses of the complexes by microspreading.

Demonstrations and discussions on the synaptinemal complex will involve the fungi Neottiella, Sordaria, Neurospora, Coprinus, the higher plants Lilium, Triticum and Zea, the insects Bombyx and Drosophila, the mouse and Homo sapiens Pairing with the synaptinemal complex in haploids, triploids and tetraploids, in achiasmatic meiosis, in inversions, sex bivalents and situations with large differences in crossing-over frequencies will be analysed. Lectures on the nucleosome structure and the biochemistry of meiosis will be presented The seventh day is intended to give students the possibility to acquire some of the techniques (know-how in formvar films, fixation, embedding, etc) in greater detail

INSTRUCTORS and INVITED SPEAKERS:

R. HOLLIDAY (London)	R. RILEY (London)
P. HOLM (Copenhagen)	H. STERN (La Jolla)
B. C. LU (Guelph)	J. THOMAS (Cambridge)
J. L PETERSEN (Copenhagen)	L TRES (Chapel Hill)
S. W. RASMUSSEN (Copenhagen)	D. von WETTSTEIN (Copenhagen)
	D. ZICKLER (Orsay)

Participants are offered free dormitory accommodations and a limited number of fellowships for assistance with travel expenses are available.

Applications containing information on current research interest should be sent **before March 10, 1979** to

Professor D. von Wettstein
Carlsberg Laboratory, Gamle Carlsberg Vej 10
DK-2500 Copenhagen Valby, Denmark

8909(D)

SIMON FRASER UNIVERSITY

MASTER OF NATURAL RESOURCES MANAGEMENT PROGRAM

Applications are invited from biologists to join the newly-established Master of Natural Resources Management Program The position is a joint-appointment between the M R M Program and the Department of Biological Sciences Teaching responsibilities will include two graduate courses in the M R.M Program (1) population, community and ecosystem ecology, and (2) biological resources and resource management models, and courses in the Department of Biological Sciences Ph D required and research experience related to resource management preferable Appointment will be made at the Assistant/Associate Professor level Appointment effective July 1, 1979 Any appointee must be a Canadian citizen or eligible for landed immigrant status All appointments are subject to budgetary restraints

Please send applications accompanied by supporting documentation and names of three referees to Dr M. L. Barker, Acting Director, Master of Natural Resources Management Program, Faculty of Interdisciplinary Studies, Simon Fraser University, Burnaby, B C V5A 1S6 Deadline for applications January 31, 1979.

8868(A)

Under the sponsorship of the European Communities, the Department de Biologie Moléculaire of the Université Libre de Bruxelles is organizing an interdisciplinary training course to take place in Rhode-St -Genèse, from September 5 to 25, 1979 on

FIDELITY OF THE REPLICATION AND EXPRESSION OF GENETIC INFORMATION

Course directors M ERRERA and M RADMAN

The course will consist of 2 weeks of practical work during which each group will work on one of the following topics

1) Fidelity assays of translation with various ribosomal mutants
2) Codon-anticodon interactions
3) In vitro assays on accuracy of DNA synthesis without and with mutagenic treatments
4) Accuracy of in vivo DNA replication: mutagenicity assays with E. coli and phage λ
5) Chromosomal effects of carcinogens and malignant transformation

Every morning of the third week results of one group will be presented and discussed with all the course participants and with invited speakers who will lecture in the afternoons on subjects related to the morning's discussions (±2 lectures each afternoon)

The working language of the course will be English

Applications for enrolment should reach the Molecular Biology and Radiobiology Course Secretariat, Commission of the European Communities, DG XII, rue de la Loi 200, B-1049 Brussels, before 16 March 1979 and be accompanied by the applicant's personal history (stating knowledge of languages), a list of his scientific publications, a letter from the head of his laboratory and a justified order of preference for the five topics mentioned above.

Further information may be obtained from the same address

8898(D)

Please mention
Nature
when replying to
these advertisements

STUDENTSHIPS

THE QUEEN'S UNIVERSITY OF BELFAST

S.R.C. RESEARCH STUDENTSHIPS

The Science Research Council is likely to offer to suitable candidates a limited number of research studentships for 1979 tenable in the Faculties of Science or Applied Science and Technology; the former includes the Departments of Pure Mathematics, Applied Mathematics and Theoretical Physics, Chemistry (Analytical, Inorganic, Organic and Physical), Industrial Chemistry, Biochemistry, Zoology, Botany, Psychology, Archaeology and History and Philosophy of Science; the latter, Engineering Mathematics, Civil, Mechanical and Industrial, Electrical and Electronic and Aeronautical Engineering Departments.

The value of these will be in accordance with S.R.C. scales and students graduating in 1979 in Great Britain (and who are not resident in Northern Ireland) are invited to write stating their particular field of interest to the Dean of the selected Faculty by January 15, 1979. 8914(F)

FELLOWSHIPS

UNIVERSITY OF YORK

DEPARTMENT OF BIOLOGY

S.R.C. RESEARCH FELLOWSHIP

Applications are invited for a Postdoctoral Research Fellowship in collaboration with Dr J. Digby and Dr R. D. Firn. The work is on the control of the different growth which causes geocurvature and subsequent autotropic straightening of shoots. The candidate should have a background in plant physiology. A knowledge of German would be an advantage.

The position is tenable for three years and will be on the salary scale £3,883 to £4,382 (under review).

Three copies of applications, including the names of three referees, should be sent by February 15, 1979 to the Registrar, University of York, Heslington, York, Y01 5DD. Informal enquiries can be made to Dr Digby or Dr Firn, Department of Biology. Please quote reference number N/6095. 8872(E)

UNIVERSITY OF OXFORD

UNIVERSITY LECTURESHIP IN PSYCHOLOGY

TUTORIAL FELLOWSHIP AT ST ANNE'S COLLEGE

Applications are invited for the above post. Stipend according to age on the scale £3,883 to £8,257 per annum. Preference will be given to candidates with interests in the fields of Language and Communication, Cognition or Perception. The post may be held in association with a tutorial fellowship at St. Anne's College (open to men and women).

Further details may be obtained from the Acting Head, Department of Experimental Psychology, South Parks Road, Oxford OX1 3UD, to whom applications (two typed copies, one from overseas applicants) including the names of three referees should be sent by January 31, 1979. 8921(E)

UNIVERSITY OF SUSSEX

SCHOOL OF BIOLOGICAL SCIENCES

POSTDOCTORAL RESEARCH FELLOWSHIP IN BIOCHEMISTRY

Applications are invited for an M.R.C. financed Research Fellowship concerning studies of the rate of calcium and cyclic AMP in the release of growth hormone and corticotropin from the adenohypophysis. Previous experience in the field of isolated pituitary cells and/or biochemical endocrinology desirable. The fellowship is available from January 1, 1979 for one year in the first instance, extendable for a further eighteen months.

Salary in the range £3,883 to £4,882 per annum (under review) on the Research Fellow grade 1A scale.

Applications, including curriculum vitae, a list of publications if any, and the names of three referees, as soon as possible please to: Dr D. Schulster, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG. 8891(E)

UNIVERSITY COLLEGE DUBLIN

DEPARTMENT OF HORTICULTURE

POSTDOCTORAL FELLOWSHIP

Applications are invited for a postdoctoral fellowship, sponsored by The National Board for Science and Technology, to study protein degradation and reutilisation in fruit trees and germinating seeds.

Applicants should have relevant research experience in the fields of horticulture, plant physiology or biochemistry.

The appointment will be for one year, initially, beginning in early 1979. The starting salary will be £3,762 per annum on an incremental scale.

Applications giving details of qualifications and experience and the names and addresses of two referees should be sent to:

Dr M. J. Hennerty,
Department of Horticulture,
University College,
Glasnevin,
Dublin, 9. 8932(E)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF PHYSIOLOGY

RESEARCH FELLOWSHIP IN EXPERIMENTAL NEUROLOGY

Applications are invited for a Research Fellow IA in the Department of Physiology. The Fellow will work on a five-year M.R.C. funded project involving the study of motor control in human subjects and other primates. Preference will be given to candidates with experience in either clinical neurology or neurophysiological research.

Salary will be on the research scale IA £3,883 to £6,555 (plus superannuation) according to age, qualifications and experience subject to maximum starting salary of £4,382.

Further details about the work may be obtained from Dr P. M. H. Rack, Department of Physiology, The Medical School.

Applications, three copies, with curriculum vitae should be sent to the Assistant Registrar, The Medical School, Birmingham B15 2TJ, by January 26, 1979. Please quote reference RF/PHY/478. 8918(E)

UMIST

CHAIRS IN CONTROL ENGINEERING AND IN APPLIED CONTROL ENGINEERING

The Chair in Control Engineering will be relinquished by Professor H. H. Rosenbrock, F.R.S., on December 31, 1978, to take up a S.R.C. Senior Fellowship. The Control Systems Centre, apart from a very small element of undergraduate service teaching, is essentially concerned with teaching and research at postgraduate level, but this pattern is not necessarily fixed for the future. The Institute recognises that the theoretical aspect of Control Engineering is heavily mathematically oriented, whilst the practical aspect is concerned with the many areas of application in industrial situations. The Control Systems Centre has so far concentrated on the middle ground, avoiding the more abstract theory and also avoiding too deep a commitment to applications. Effective links have been established with the Department of Mathematics to further the study of the theoretical aspects, and the Centre has also successfully collaborated with Engineering and other Departments in projects involving application. In recognition of the continuing development and expansion of the field the Institute has concluded that it should support two Chairs in this area. The Chair in Control Engineering will be filled by someone with an interest in theory, and a knowledge of practice.

The new Chair in Applied Control Engineering will be expected to develop further the existing active liaison with some of the Engineering and other Departments, and candidates should have wide experience in the applications of control to operations of industrial or commercial significance.

For both Chairs candidates are sought who have a firm commitment to research, who have proven teaching ability and who are capable of providing leadership and encouragement to the other members of staff, especially in research. By virtue of their scholarship and scientific interests they should be able to engender collaborative ventures with industry, government agencies, etc., and to command financial support from S.R.C., industry, etc.

Applications should be received as soon as possible after the appearance of this advertisement. Salary will be in the Professorial range with a minimum of £9,443 per annum (plus a cost of living increase still to be negotiated) and a further increase due in October 1979.

Further particulars and application forms may be obtained from the Registrar, UMIST, P.O. Box 88, Manchester M60 1QD, by quoting reference CSC/222/AI for Control Engineering and CSC/223/AI for Applied Control Engineering.

Informal enquiries may be made to the Registrar or to Professor H. H. Rosenbrock with a view to a preliminary informal visit. 8911(A)

ODENSE UNIVERSITY

Institute of Biochemistry

POSTDOCTORAL RESEARCH FELLOWSHIP

Lipid Biochemistry

Applications are invited for the above post for a maximum of three years to work in a research group engaged in studies of mammary gland lipid metabolism. The research fellowship has been granted to investigations on the role of intracellular fatty acid binding proteins in the regulation of fatty acid synthesis and development of methods for measurement of cellular acyl-CoA concentrations. Research experience in lipid metabolism, methods of lipid analysis and protein purification is desirable. The position is available immediately. Salary range: Danish kroner 7.856,36 to 12.998,59 per month plus bonus.

Further information can be obtained from Dr Jens Knudsen, Institute of Biochemistry, Odense University, Campusvej 55, 5230 Odense M, Denmark.

Applications should include a curriculum vitae and documentation of academic qualifications. Applications must be marked "stilling nr. 637" and should reach the Administration, Odense University, journalkontoret, Campusvej 55, 5230 Odense M, Denmark, not later than January 16, 1979. 8873(E)

CONFERENCES

EMBO WORKSHOP

European Molecular Biology Organisation

Carlsberg Laboratory Copenhagen

June 11–16, 1979

Chloroplast membrane polypeptides

Organisers: R. J. Ellis (Coventry) and D. von Wettstein

This workshop is concerned with the functional characterisation, the biosynthesis and the genetics of chloroplast membrane polypeptides. Emphasis will be placed on comparing and coordinating efforts to separate and characterise the thylakoid polypeptides in organisms amenable to genetic analysis.

THE PARTICIPANTS WILL INCLUDE:

K. APEL (Freiburg)
C. ARNTZEN (Urbana)
P. BENNOUN (Paris)
R. J. BERZBORN (Bochum)
N. BISHOP (Corvallis)
P. BÖGER (Konstanz)
N.-H. CHUA (New York)
R. DOUCE (Grenoble)
N. GILLHAM (Durham)
J. GRAY (Cambridge)
K. W. HENNINGSEN (Copenhagen)
G. HØYER-HANSEN (Copenhagen)
C. J. LEAVER (Edinburgh)
O. MACHOLD (Gatersleben)
R. MALKIN (Berkeley)
B. LINDBERG MØLLER (Copenhagen)
N. NELSON (Haifa)
J. A. SCHIFF (Waltham)
G. H. SCHMID (Cadarache)
D. SIMPSON (Copenhagen)
J. P. THORNBER (Los Angeles)
L. P. VERNON (Provo)
K. WEGMAN (Tübingen)

The workship is intended for scientists actively working in this field. Applicatinns containing information on past and present research activity should be sent **before March 1, 1979** to

Professor D. von Wettstein
Carlsberg Laboratory, Gamle Carlsberg Vej 10
DK-2500 Copenhagen Valby, Denmark

8910(C)

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Applications to attend this Summer School should be made to reach Dr B. K. Tanner, Department of Physics, Durham University, Science Laboratories, South Road, DURHAM DH1 3LE, U.K. by January 31, 1979.

8897(D)

ASSISTANTSHIPS

QUEEN MARY COLLEGE

University of London
APPLIED MATHEMATICS DEPARTMENT

Applications are invited for a one-year

POSTDOCTORAL RESEARCH ASSISTANTSHIP

to begin not later than March 31, 1979, for work with Drs S. Simons and I. P. Williams on the optical properties of multicored interstellar grains.

Salary in range £4,359 to £5,342 per annum (including London Allowance).

Please apply by letter to the Registrar (N), Queen Mary College, Mile End Road, London E1 4NS, enclosing curriculum vitae and names and addresses of two referees.

8931(P)

UNIVERSITY COLLEGE LONDON

RESEARCH ASSISTANTSHIP

A short-term research assistantship is available in the Department of Zoology, starting in March 1979.

Applicants with experience of either cell electrophoresis or the production of hybridomas are invited to write to Professor N. A. Mitchison, University College London, Gower Street, London WC1E 6BT, before January 19, 1979.

8906(P)

ASSISTANTSHIPS (continued)

UNIVERSITY OF SOUTHAMPTON

SCHOOL OF BIOCHEMICAL AND PHYSIOLOGICAL SCIENCES

GRADUATE RESEARCH ASSISTANTSHIP

Applications are invited for appointment as a GRADUATE Research Assistant in the BIOCHEMISTRY GROUP of this School, to work with Dr D. P. Bloxham on the applications of thiosulphonate reagents to problems in protein structure and function. Applicants should have a good honours degree in biochemistry or chemistry and should have an interest in enzymology. The appointment, which is S.R.C. supported, will be made as soon as possible and is initially for one year, renewable for a further two years.

The salary will be within £3,884 to £4,883 per annum (under review) on Scale 1B for Research and Analogous Staff; U.S.S. benefits.

Applications giving date of birth, curriculum vitae and the names and addresses of two referees, should be sent to Mrs P. Vaughan-Smith, The University, Southampton SO9 5NH, by January 20, 1979, quoting reference 1043/R/Na.

8889(P)

UNIVERSITY OF DURHAM

DEPARTMENT OF CHEMISTRY

Applications are invited for chemists or chemical physicists for an S.R.C. Postdoctoral Senior Research Assistantship for 17 months from as soon as possible to work in collaboraton with Dr J. Yarwood on the interaction and dynamics of hydrogen bonded systems in the liquid phase. These studies which include infrared, far-infrared, Raman and neutron scattering studies are also supported by a N.A.T.O. research grant and would be carried out at Durham, the Technical University, Braunschweig and at A.E.R.E. Harwell. Some experience in one branch of molecular spectroscopy is essential and an interest in the chemical physics of molecular motions and interactions in the liquid phase would be at advantage.

Initial salary £3,883 or £4,133 per annum on National Research Range 1A plus superannuation.

Applications (three copies) naming three academic referees should be sent by January 22, 1979, to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE.

8904(A)

UNIVERSITY OF DURHAM

DEPARTMENT OF APPLIED PHYSICS AND ELECTRONICS

Applications are invited for an S.R.C.

POSTDOCTORAL RESEARCH ASSISTANTSHIP

tenable for three years from early 1979. Applicants should have qualifications in physics, materials science, electronics or a related subject.

The successful applicant will work on Electroluminescence in Zinc Sulphide in the group directed by Dr J. Woods, and will be concerned with the fabrication and evaluation of light emitting diodes and larger area devices.

Initial salary £3,883 or £4,133 per annum on National Research Range 1A, plus superannuation.

Applications (three copies) naming three referees, should be sent by January 8, 1979, to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained.

8878(P)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF APPLIED BIOCHEMISTRY AND NUTRITION
FOOD SCIENCE LABORATORIES

A.R.C. RESEARCH ASSISTANTSHIP

tenable from January 1979.

Applications are invited for the post to study the dynamics of starch gelatinisation using one or more novel physicochemical techniques. Candidates may register for a Ph.D. degree but should preferably have a good honours degree in Chemistry, Physics or some related subject.

Salary £3,384 to £3,883.

Applications, naming two referees, should be sent as soon as possible to: J. M. V. Blanshard, Department of Applied Biochemistry and Nutrition, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD, from whom further details may be obtained.

8871(P)

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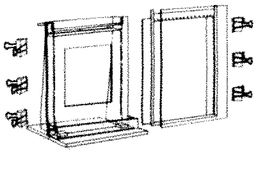

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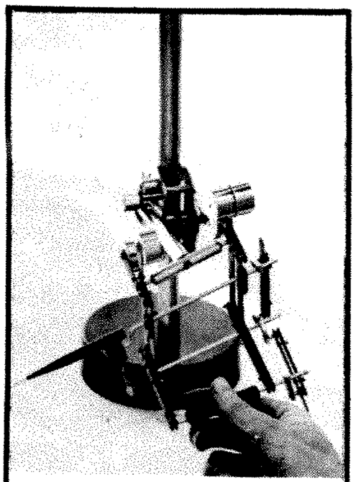

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